OFFICE OF NAVAL RESEARCH

CONTRACT N00014-94-C-0149

TECHNICAL REPORT 97-03

EFFECTS OF CARDIOPULMONARY BYPASS ON HEMOSTASIS

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8 JULY 1997

Effects of cardiopulmonary bypass on hemostasis

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INTRODUCTION

The vast majority of cardiac surgical operations are performed with cardiopulmonary bypass (CPB). Blood contact with the extracorporeal circuit used during CPB elicits a wide spectrum of pathophysiological changes that affect a variety of organ systems. In a broad, sense the hematologic changes brought about by CPB are probably the most important of these pathophysiologic states because they result in the most pronounced clinical abnormality, increased postoperative bleeding, and because they affect several of the abnormal clinical manifestations of other organ systems (e.g., increased capillary permeability leading to respiratory abnormalities). It has long been recognized that CPB results in abnormal hemostasis that leads to increased postoperative bleeding. The exact nature of this hemostatic abnormality remains the subject of intense investigation, although platelet function abnormalities and hyperfibrinolysis have been shown to contribute to it significantly. The purpose of this chapter is to provide a description of our current knowledge of the hematologic changes observed in the course of CPB, to review our understanding of the nature of the hemostatic defect elicited by contact with the extracorporeal circuit, and to address clinical issues related to postoperative blood loss, the most important clinical outcome of these pathophysiologic states.

EFFECTS OF CPB ON BLOOD ELEMENTS

Platelets and the vessel wall

Normal platelet physiology (Fig. 1)

Platelets are essential for normal hemostasis. The main functions of platelets are adhesion to damaged vessel walls, aggregation to form a platelet plug, and promotion of fibrin clot formation. The mechanisms of platelet activation are reviewed in detail in other chapters; the following serves as a brief summary to provide background for the clinical abnormalities of CPB.

Platelet adhesion is mediated primarily by the adhesive molecule von Willebrand factor (vWF), which binds both to a specific receptor on the platelet surface [glycoprotein (GP)Ib-IX complex] and to exposed subendothelial components [1,2]. Platelet-to-platelet association (aggregation) is primarily mediated by fibrinogen binding to its receptor on the platelet surface GPIIb-IIIa complex [3]. Normal circulating platelets are in a resting state and bind neither plasma vWF nor plasma fibrinogen. *In vitro* the cationic antibiotic ristocetin induces binding of vWF to its receptor on GPIb [1], but the *in vivo* analog of ristocetin remains uncertain. Shear stress and/or fibrin (monomer) interacting with vWF may serve as the physiologic stimulus to initiate GPIb-vWF interaction. Thrombin and other physiologic platelet agonists (e.g., adenosine 5-diphosphate (ADP), epinephrine) induce exposure of the fibrinogen receptor on the platelet surface GPIIb-IIIa complex [3]. These agonists also stimulate platelets to change shape, secrete the contents of their granules (e.g., β-thromboglobulin (β-TG), platelet factor 4 (PF4), thrombospondin), and to aggregate. Secreted thrombospondin binds to a receptor on the platelet surface membrane, as well as to fibrinogen, thereby stabilizing platelet aggregates [4]. P-selectin [5], also known as CD62P [and previously known as granule membrane protein-140 (GMP-140) [6] and platelet activator-dependent granule to external membrane (PADGEM) protein [7], is a component of the α-granule membrane of resting platelets that is only expressed on the platelet plasma membrane after platelet activation and secretion [8]. Platelet surface expression of P-selectin is, therefore, marker of platelet degranulation. Platelet surface P-selectin mediates the adherence of degranulated platelets to leukocytes in thrombi [9-11] and induces the expression of tissue factor on monocytes [12]. In contrast to its effect on P-selectin and the fibrinogen receptor on the GPIIb-IIIa complex, thrombin decreases the platelet surface expression of the vWF receptor on the GPIb-IX complex [13-16].

Time-course of changes in platelet parameters during and following CPB

Bleeding time

The bleeding time is markedly prolonged during hypothermic CPB [17-19]. It normalizes within 24 hours postoperatively [17]. The operator dependence of the measurement of the bleeding time and its temperature-dependence [20] have contributed to a lack of consistency in the data reported. As evidenced

from uniform measurements made in 87 patients undergoing uncomplicated myocardial revascularization procedures, systemic anticoagulation with 3mg/kg of heparin prior to the institution of CPB elicits a modest but significant prolongation of the bleeding time (Fig. 2). The bleeding time is markedly prolonged during CPB and remains so for 2 hours after bypass. Although it essentially returns to normal between 2 and 24 hours after CPB, the bleeding time may not normalize until 72 hours postoperatively (see Fig. 2). The marked prolongation of the bleeding time in the course of CPB and its subsequent reversal suggest that platelet dysfunction is a primary culprit in the hemostatic defect induced by CPB.

Platelet count

Decrease in the platelet count is consistently observed during and following CPB [17, 18, 21-23] (Table 2); it occurs as early as 5 minutes following the institution of CPB [23] and reaches its nadir by 25 minutes [18]. The platelet count remains depressed throughout the postoperative period (see Table 2) and may continue to be depressed for several days after the bypass procedure [17, 23].

Hemodilution and platelet adhesion to synthetic surfaces are the two primary contributors to the thrombocytopenia observed during CPB. During extracorporeal circulation for cardiac surgery, dilution of blood occurs as a result of priming the extracorporeal perfusion system with either crystalloid or colloid solutions. This hemodilution is generally considered to be the major cause of thrombocytopenia during CPB [21, 22]. However, the degree of thrombocytopenia observed during and following CPB is more severe than that which might be expected from hemodilution alone [18, 24]. A loss of platelets during CPB occurs that is proportional to the flow rate and the surface area of the extracorporeal circuit [25]. Platelets have been shown by scanning electron microscopy to adhere to extracorporeal synthetic surfaces [26]. Fibrinogen appears to be the most important cofactor in platelet adhesion to synthetic surfaces [27], as it is for platelet aggregation [3]. Plasma fibrinogen is preferentially adsorbed onto synthetic surfaces [28, 29], and platelet reactivity with these surfaces has been reported to be directly proportional to the adsorbed fibrinogen concentration [30], although this supposition has been disputed by one group of investigators [31]. The mechanism(s) by which platelets are initially activated within extracorporeal perfusion systems is not completely clear, but possible causes include direct surface contact, thrombin formation, and ADP release. Thrombin, which is generated in small amounts despite the presence of heparin during CPB surgery [32], is adsorbed onto synthetic surfaces [33] and likely binds to the adsorbed fibrinogen on the extracorporeal surface. ADP is stored in platelet dense granules and is released both by platelet lysis and platelet activation, hemolysis of red cells also releases ADP.

Platelet activation results in exposure of fibrinogen binding sites on the GPIIb-IIIa complex [34] and permits binding to fibrinogen molecules previously adsorbed onto the surface [35]. Gluszko and colleagues [31] have shown that exposure of fibrinogen receptors associated with the GPIIb-IIIa complex contributes to platelet consumption during CPB. These investigators [31] demonstrated that patients with Glanzmann's thrombasthenia (an inherited deficiency of the GPIIb-IIIa complex) had reduced CPB-induced thrombocytopenia, whereas patients with the Bernard-Soulier syndrome (an inherited deficiency of the GPIb-IX-V complex) did not.

Activated platelets, in addition to adhering to the synthetic surfaces of the CPB tubing, are also more likely to adhere to injured vascular surfaces and to deposit in the heart after cardioplegic arrest [36]. The concept that platelet activation and adherence are important in the etiology of thrombocytopenia during CPB is supported by findings that infusion of prostaglandin E_1 (PGE_1), prostacyclin (PGI_2), iloprost, or dipyridamole (drugs which inhibit platelet activation) during CPB can result in a marked reduction in platelet adherence to the synthetic surfaces, maintenance of platelet counts at near-normal levels, and reduction in postoperative blood loss [31, 37-44]. An adsorbed protein layer that reduces the affinity of synthetic surfaces for platelets may eventually form [45, 46]. Although the exact physiologic basis for this process, termed passivation [21], remains unclear, support for the concept derives from studies with PGI_2 [37, 38]. When PGI_2 was used to inhibit platelets during two hours of recirculation in a membrane oxygenator system, platelet activation was inhibited only during the first hour [37, 38]. PGI_2 is extremely unstable in plasma, and after 1 hour the recirculated platelets regain their ability to aggregate in the presence of ADP and epinephrine, yet do not react with the synthetic surface [37, 38].

Oxygenators, and to a lesser extent, filters contain the largest surface areas in contact with blood, and therefore are the most prominent sites of platelet deposition [21]. The degree of thrombocytopenia observed during CPB is related more to the turbulence, flow rate, and amount of suction in the circuit [25, 47, 48] than to the type of oxygenator employed [18,49].

Three less common causes of thrombocytopenia during CPB are disseminated intravascular coagulation (DIC), heparin-induced thrombocytopenia, and cyanotic congenital heart disease. Although probably uncommon, as distinct from primary fibrinolysis [50], DIC is a cause of thrombocytopenia and possibly of increased bleeding following CPB [51,52]. DIC is rarely encountered during CPB but is more likely to occur later, in association with sepsis or low cardiac output [21]. Heparin-induced thrombocytopenia is discussed later in this chapter and in Chapter 29. The mechanism of the association between cyanotic congenital heart disease and thrombocytopenia [53, 54] is unclear.

Mean platelet volume and platelet mass

The mean platelet volume (MPV) decreases significantly after the institution of CPB and reaches its nadir approximately 2 hours after discontinuation of bypass (see Table 2). There is a progressive and significant increase in MPV between 2 and 72 hours postoperatively, accompanied by a significant rise in platelet mass [18], suggesting that larger platelets are selectively removed during the extracorporeal circulation [18, 55]. This is an important finding in light of the fact that platelet size has been shown to relate directly to platelet function , with larger platelets being more hemostatically competent than smaller platelets [56]. An increase in MPV between 2 and 72 hours postoperatively, accompanied by a relatively stable platelet count, suggests that a postoperative release of large platelets into the peripheral circulation is responsible in part for the improvement in the bleeding time observed during this period.

α-Granule release

When platelets adhere to synthetic surfaces they are activated and the contents of their α-granules are released into the circulation. Plasma levels of β-TG and PF4, both of which are contained within α-granules, are markedly increased during and immediately following CPB [17, 57-61]. Levels return to normal within the 24-hour postoperative period (Table 2) [17, 18]. These changes in the plasma levels of platelet-specific proteins may reflect an initial, irreversible activation and lysis of a relatively small number of platelets, which are removed from the circulation within the first 24 hours following CPB[62].

Thromboxane B_2 and 6-keto $PGF_{1\alpha}$

Studies conducted in the baboon and in humans in which thromboxane B_2 (the stable derivation product of thromboxane A_2) and 6-keto $PGF_{1\alpha}$ (the stable derivation product of prostacyclin) were measured in blood shed from the skin at the site of the bleeding time have yielded valuable information on platelet function [63-65]. In patients undergoing CPB, a marked reduction in the level of thromboxane B_2 in the shed blood has been observed soon after the institution of CPB, and is indicative of platelet dysfunction (Table 2) [18, 20]. Within 2-24 hours after the discontinuation of CPB, the level of thromboxane B_2 in the shed blood is significantly increased, while the systemic plasma level of thromboxane B_2 is decreased (Table 2) [18, 19]. The increase in the shed blood thromboxane B_2 postoperatively is not a reflection of changes in the plasma concentration of this product, but, rather, the progressive improvement in platelet function postoperatively, which is paralleled by an improvement in the bleeding time and an increase in the MPV. Changes in the shed blood 6-keto $PGF_{1\alpha}$, both during and after CPB (Table 2), are generally opposite to those observed in the shed blood thromboxane B_2, and are probably reflective of the systemic

changes in plasma 6-keto PGF1α [18].

Platelet aggregation

Blood samples obtained from patients during CPB have shown markedly reduced platelet aggregation in response to *in vitro* stimulation with various agonists [22, 49, 66-70]. However, the *in vitro* nature of this test, the wide variability in the reported responses to the various agonists, and the wide variability in the data reported by various investigators underscore the unreliability of this measurement in the quantification of the degree of platelet dysfunction elicited by CPB.

Role of platelets in the hemostatic defect induced by CPB

There is a marked prolongation of the bleeding time during CPB (17-19). Three possible causes of a marked prolongation of the bleeding time are: reduced von Willebrand factor, thrombocytopenia, or a platelet function defect. During CPB the plasma level of von Willebrand factor is normal [71] or increased [72,73]. The modest degree of thrombocytopenia that occurs during CPB [19] is insufficient to account for the marked prolongation of the bleeding time [74]. Therefore, the marked prolongation of the bleeding time during CPB is mainly the result of a platelet function defect.

The cause(s) of the platelet function defect during cardiopulmonary bypass is not entirely clear. Decreases in platelet aggregation in response to ADP, epinephrine, and collagen, as well as abnormalities in platelet release, have been observed preoperatively in patients with cyanotic congenital heart disease [75,76]. Possible causes of the platelet function defect are listed in Table 1. Circulating fibrin(ogen) degradation products are present in the majority of patients undergoing CPB surgery [50], and may interfere with platelet function [77]. A direct correlation between the plasma levels of D-dimer and the magnitude of platelet dysfunction has been reported in patients undergoing cardiac surgery [18]. Improved platelet aggregation correlated with lower levels of D-dimer after cardiopulmonary bypass [78], and the bleeding time at two hours following cardiopulmonary bypass correlated with the corresponding level of plasma D-dimer. A correlation has been observed between high concentrations of denatured plasma proteins and reduced platelet function [79] during CPB, particularly in bubble oxygenator perfusion systems [80,81] in which plasma proteins are denatured.

The variability in the reported defects in platelet function during and after bypass results in part from differences in equipment and techniques. However, methodological problems may also be involved. During the process of separating platelets from whole blood for functional assays, the platelets are

susceptible to membrane alterations [82] and to *ex vivo* activation. The popular use of plasma assays of the secretion products of platelet α-granules (PF4 and β-TG) to study the platelet defect in CPB is questionable because 1% platelet secretion as a result of *in vitro* handling may cause as much as a 30-fold increase in the plasma level of PF4 [83]. Moreover, plasma assays of PF4 and β-TG reflect not only the number of circulating activated platelets but also lysed platelets and non-circulating activated platelets adherent to synthetic surfaces on the vessel walls.

To circumvent these problems with regard to platelet function testing, whole blood flow cytometric assays that do not involve any separation or manipulation of platelets have been developed [84]. As determined by these assays [19], CPB results in: 1) markedly deficient platelet reactivity in response to an *in vivo* wound (Fig. 3), 2) normal platelet reactivity *in vitro* (Figure 4), 3) no loss of the platelet surface GPIb-IX or GPIIb-IIIa complexes (Figure 5), and 4) a minimal number of circulating activated platelets (Figure 4). These data [19] suggest that the "platelet function defect" of CPB is not a defect intrinsic to the platelet, but is an extrinsic defect such as an *in vivo* lack of availability of platelet agonists. The near universal use of heparin during CPB is likely to contribute substantially to this defect via its inhibition of thrombin, the preeminent platelet activator [85-87].

There are two distinct effects of heparin on platelet function during CPB [19, 88, 89]. First, heparin augments platelet activation in whole blood exposed to an exogenous platelet agonist *in vitro* (Fig. 4). Second, heparin suppresses platelet activation *in vivo*, as demonstrated by abrogation of the activation-induced increase in platelet surface P-selectin (Fig. 3), prolongation of the bleeding time [19, 88], and reduction in the level of shed blood thromboxane B_2 [19, 88]. These effects are presumably mediated via inhibition of endogenous thrombin [19]. Thus, although heparin augments the activatibility of platelets, the platelets are not in fact activatible *in vivo* during CPB because thrombin, the preeminent agonist [85-87], is unavailable. In addition to heparin, other extrinsic factors such as hypothermia [18, 20, 90] and fibrinolytic activity [18, 75] contribute to the platelet function defect associated with CPB. (These factors are discussed below.)

Earlier studies using washed platelets suggested a degree of reduction in either platelet surface GPIb-IX [91] or GPIIb-IIIa complexes [92,93] during CPB. However, the differences between these studies and whole blood studies [19, 94] are probably the result of artifactual *in vitro* changes in platelet membrane receptors caused by the separation procedures required for the isolation of platelets. Although some whole blood studies show modest reductions of either platelet surface GPIb-IX [13, 95] or GPIIb-IIIa complexes [95], these changes cannot explain the marked prolongation in the bleeding time during CPB because heterozygotes for Bernard-Soulier syndrome and Glanzmann's thrombasthenia (who lack

50% of platelet surface GPIb-IX and GPIIb-IIIa complexes, respectively) do not have a bleeding diathesis and have a normal bleeding time.

In view of the only modest increase in platelet surface P-selectin during CPB [19, 95,96], the more profound increase in the plasma concentrations of soluble P-selectin, β-TG, and PF4 [17, 22, 97] during CPB may reflect one or more of the following: I) platelet surface expression of P-selectin results in the formation of either circulating monocyte-platelet aggregates or neutrophil-platelet aggregates, resulting in loss of circulating single platelets expressing surface P-selectin [96]. ii) circulating degranulated platelets rapidly lose surface P-selectin to the plasma pool, but continue to circulate and function [98]. iii) non-circulating, degranulated platelets adhere to synthetic surfaces or the vessel wall. iv) platelet lysis *in vivo* [62] or *in vitro.* and/or v) artifactual *in vitro* degranulation and secretion as a result of separation of plasma from platelets before the performance of the assays [99].

CPB has also been reported to be associated with a modest increase in the plasma concentration of platelet-derived microparticles [13, 100], apparently as a result of turbulence and shear stress.

Fluid phase: coagulation and fibrinolysis

Time-course of changes in plasma proteins during and following CPB

Most of the plasma proteins are adsorbed to the CPB circuit in small and inconsequential amounts. Fibrinogen, by contrast, is preferentially adsorbed to synthetic surfaces and is the dominant protein on these surfaces [28,29]. The institution of CPB elicits nearly a 50% decrease in the concentration of plasma proteins, mainly as a result of hemodilution. Because of the complexity of cardiac surgery and its demand for frequent administration of various types of fluids and blood components, the exact contribution of hemodilution to the observed concentration of the various plasma proteins during and following CPB is difficult to ascertain. As reflected by the changes in hematocrit (Table 2), hemodilution is most pronounced within minutes after the institution of CPB; it is sustained, to a lesser degree, for several days postoperatively. Protein concentrations, which fall during CPB and remain low throughout the initial postoperative days, may be reflective of hemodilution and not of sustained intraoperative consumption.

Plasma protein changes during and following CPB should be interpreted in light of the dilutional state. However, it is not appropriate to correct for hemodilution by simply relating the plasma protein concentration to the corresponding hematocrit because the proportion of plasma to whole blood is

different from that of the red cells to whole blood. Correcting for hemodilution during and following CPB without due consideration of this fact is another source of difficulty in the interpretation of published plasma protein concentration data. The following is a more appropriate formula for use in the calculation of the concentration of a plasma protein independent of the dilutional effect elicited by the institution of CPB:

$$DCP = BP\ [BL\ HCT/(BP\ HCT - BL\ HCT)/(1 - BL\ HCT)]$$

where DCP = dilution-corrected protein concentration during CPB, BP = actual protein concentration during CPB, BL = baseline (prebypass) value, HCT = hematocrit.

Oncotic and opsonic proteins

Total protein concentrations reflect the dilutional state observed during and following CPB [18]. Albumin is minimally adsorbed to the extracorporeal circuit. During the period between 2 and 72 hours postoperatively, albumin remains depressed, paralleling the hematocrit (see Table 2). The opsonic proteins (IgG, IgM, C3, and fibronectin) also decrease significantly during and following CPB; they remain depressed for at least 3 days postoperatively (Table 2). C3 increases significantly during the 24-72-hour post-CPB period, but it does not return to its baseline value (Table 2). The decrease in the opsonic proteins during and following CPB is dilutional, but has also been attributed to generalized opsonic consumption during prolonged CPB [101-102], fibronectin-mediated removal of macrocellular aggregates by the reticuloendothelial system [103], protein degradation by proteolytic enzymes [104], and cold-induced precipitation of fibronectin with fibrinogen [105].

Coagulation and fibrinolytic proteins

Plasma fibrinogen levels are elevated above normal in patients with heart disease [106-108]. Likewise, they are elevated preoperatively in patients undergoing cardiac surgery [18, 57]. Two hours following CPB, fibrinogen levels are decreased, but between 2 and 72 hours postoperatively there is a progressive increase in plasma fibrinogen, resulting in levels that are significantly higher than baseline (Table 2). Likewise, there is a progressive increase in the concentrations of factor VIII and vWF between 2 and 72 hours following CPB, resulting in levels that are significantly higher than baseline (Table 2). Thus, unlike the opsonic proteins which remain depressed for a few days postoperatively, the coagulation proteins increase well above baseline during this period [18, 109-112].

Fibrinolytic activity increases significantly during and following CPB [113-124], and contributes to increased postoperative blood loss [50, 79, 113,116, 119, 125]. Fibrinolytic activity is actually observed shortly after systemic heparinization before the institution of CPB. Heparin induces a significant rise in plasma plasmin activity [88, 121], which is sustained (at a lower level probably as a reflection of hemodilution) throughout the duration of CPB. Plasmin activity returns to normal at the completion of CPB. α_2-antiplasmin, a specific inhibitor of plasmin, is also affected by systemic heparinization prior to institution of CPB. Both systemic heparinization and the institution of CPB effect a progressive decrease in the antiplasmin activity that is sustained throughout the first 24 hours postoperatively. The antiplasmin activity during bypass is significantly lower than that expected on the basis of hemodilution alone [121]. While plasmin activity returns to normal immediately after discontinuation of CPB, antiplasmin levels do not return to normal before 48-72 hours postoperatively.

The concentration of tissue-type plasminogen activator (t-PA) increases during CPB and returns to normal rapidly thereafter [114, 117, 119]; it is not affected by systemic heparinization. This suggests that CPB is a major stimulus for the release of t-PA from the vascular endothelium, in addition to the other known stimuli such as exercise, hypotensive shock, pharmacologic agents, and protein C [126]. Plasma plasminogen and antithrombin III decrease significantly during CPB and remain depressed from their baseline levels throughout the first 72 hours postoperatively (Table 2). They parallel the changes in hematocrit and are probably reflective of the dilutional state [122].

Fibrin(ogen) degradation products (FDPs) and D-dimer increase during and following CPB [115, 116, 120, 123]. At the completion of CPB, the FDP level is markedly reduced and no FDPs are detected beyond 2 hours following CPB. D-dimer, a product of the degradation of crosslinked fibrin, increases after the administration of protamine and reaches its peak 2 hours following the discontinuation of CPB (Table 2).

Role of fibrinolylic and coagulation factors in the hemostatic defect induced by CPB

There are a number of pathways that could lead to increased fibrinolysis during CPB surgery [127, 128]. The contact of blood with a large artificial surface leads to activation of the contact phase of coagulation and kallikrein generation (see Chapter 5). Kallikrein directly, and indirectly via bradykinin, stimulates release of t-PA from endothelial cells [127, 128]. Kallikrein can also convert the inactive zymogen prourokinase into urokinase. Release of t-PA from endothelial cells during CPB may be stimulated by the elevated levels of thrombin, epinephrine, angiotensin II, leukotrienes, and hypoxia [127]. Heparin can

also induce a significant rise in plasma plasmin activity and FDPs before the institution of CPB [88, 89, 121]. In addition to the fibrinolytic state per se, circulating FDPs can interfere with thrombin activity, fibrin polymerization, and platelet function [50]. Blood in the pericardial cavity activates the extrinsic coagulation pathway [129] and the fibrinolytic system [130]. Hyperfibrinolysis resulting from stasis of blood and clots in the pericardial cavity [130] can produce a vicious cycle leading to increased blood loss in the immediate postoperative period and prompting a return of the patient to the operating room for control of bleeding. Often no discrete bleeders are found in these patients on re-exploration, but evacuation of blood clots from the pericardial cavity will lead to the cessation of excessive postoperative bleeding. That increased fibrinolysis plays an important role in the genesis of the CPB-induced hemostatic defect is confirmed by studies which have related the blood levels of products of fibrinolysis to the magnitude of the post-CPB blood loss [18, 78], and by numerous placebo-controlled studies which have demonstrated a remarkable efficacy of antifibrinolytic agents in the reduction of post-CPB blood loss (see section on postoperative blood loss below).

The reductions in plasma levels of coagulation factors during CPB surgery are primarily due to hemodilution [17, 131]. Only factor V levels decrease to a level lower than that predicted by dilution alone [17, 22]. For all coagulation factors, including factor V, levels observed during CPB surgery rarely fall into a range in which hemostasis would be compromised [18, 22, 50, 51, 122].

Investigators have reported conflicting results regarding the concentration of vWF during and following CPB [18, 71-73, 132] As shown in Table 2, vWF decreases during CPB but not to levels below those considered adequate for hemostasis [18, 132]. Preoperatively, high-molecular-weight multimers of vWF may be selectively deficient in patients with valvular heart disease and noncyanotic congenital heart disease [132, 133].

The immune-inflammatory response to CPB

The activation of platelets, and the fibrinolytic and coagulation sytems during CPB is part of a "whole body inflammatory response" [134,135] that has been attributed to CPB, and which also includes the activation of complement and white blood cells, and the generation of inflammatory mediators such as TNFα, interleukin-6, and interleukin-8 [136-141]. In the course of cardiac surgery, complement is activated via both the classical and the alternative pathways. Blood contact with the artificial surfaces initiates complement activation through the alternative pathway. The administration of protamine to reverse the heparin effect after the discontinuation of CPB results in the formation of immune complexes

that trigger complement activation through the classical pathway. Complement activation results in complex adverse sequelae that include the activation and aggregation of granulocytes. For example, blockade of C5a and C5b-9 generation inhibits leukocyte and platelet activation during extracorporeal circulation [136]. A detailed description of the effect of artificial surfaces, including CPB, on complement activation, leukocyte function, and inflammatory mediators is provided in Chapter 42.

HYPOTHERMIA: ITS ROLE IN HEMOSTASIS DURING AND FOLLOWING CPB

Over the years, surgeons have intuitively recognized that hypothermia tended to increase bleeding in the surgical patient and that rewarming the patient improved hemostasis. Until recently, however, patient studies that elucidate the effect of hypothermia on hemostasis have been scarce. Canine studies have demonstrated that hypothermia at a temperature of 20°C causes thrombocytopenia, sequestration of platelets in the hepatic sinusoids, and a marked decrease in collagen-induced platelet aggregability [142, 143]. In addition, hypothermia in the dog causes a marked activation of the fibrinolytic system [143]. In the baboon, local hypothermia causes a significant increase in the bleeding time and a decrease in the concentration of thromboxane B_2 in the blood shed from the site of the measurement of the bleeding time [63]. These changes are completely reversed with rewarming [63]. Rewarming beyond 37°C does not elicit any further changes in bleeding time or the shed blood thromboxane B_2.

These observations in the hypothermic baboon have been confirmed in a clinical study of 25 patients undergoing CPB with systemic hypothermia (25°C) [20]. In these patients, one arm was kept warm with a water-jacketed cuff throughout the intraoperative and postoperative periods while the temperature in the other arm reflected the systemic changes. As demonstrated in Figure 6, the bleeding time was significantly prolonged in the cold arm compared to the warm arm; likewise, the concentration of shed blood (from the site of the measurement of the bleeding time) thromboxane B_2 was significantly lower in the cold arm than in the warm arm. These reversible changes in platelet function provided, for the first time in humans, a definitive demonstration of the effect of hypothermia on hemostatic parameters in the course of CPB. These data, along with recent data demonstrating relationships between temperature and postoperative blood loss (see below), underscore the importance of adequate rewarming following CPB to prevent platelet dysfunction and to reduce blood loss following CPB.

The effect of moderate and profound hypothermia on the hemostatic mechanism in humans cannot be safely investigated without the institution of CPB. Hence it is difficult in clinical studies to differentiate between the effects of hypothermia and the effects of CPB *per se* on the hemostatic

mechanism. However, it has been recently reported that hypothermia reversibly inhibits human platelet activation in normal volunteers in *vitro* and *in vivo* [90]. These results suggest that rewarming a hypothermic bleeding patient can reduce the need for the less safe alternative of transfusion of platelets and other blood components. Systemic hypothermia, hemodilution, and the administration of heparin during CPB have been shown to protect the patient somewhat from the adverse effects of complement activation by reducing both the generation of C3a/C5a and the subsequent cellular response of neutrophil activation [144].

CLINICAL CONSIDERATIONS

Anticoagulation during CPB

Systemic anticoagulation with heparin

Anticoagulation with heparin is central to the conduct of CPB. As described in Chapter 55, heparin elicits its anticoagulant effect by catalyzing the action of antithrombin with a resultant inhibition of thrombin and factors IXa, Xa, and XIa activities. The anticoagulant response to heparin is influenced by platelets, fibrin, vascular surfaces, and plasma proteins [145]: it is also influenced by hypothermia and hemodilution. The response is variable among patients and is disproportionately dependent on the dose and the duration of the treatment [145]. Patients receiving intravenous infusions of heparin prior to cardiac surgery require larger-than-usual doses of heparin to achieve adequate anticoagulation during CPB. The plasma biologic half-life of an intravenous injection of heparin is not uniform but is dose-dependent [146, 147]; for a dose of 100 U/kg (i.e 1 mg/kg), it is 56 minutes, while for a dose of 400 U/kg (i.e 4 mg/kg), it is 152 minutes [147]. Intravenous nitroglycerin has been considered to induce heparin resistance [148, 149], but this is still uncertain.

Heparin is administered prior to the institution of CPB in an initial intravenous dose of 250-300 U/Kg. The activated clotting time (ACT) is the most widely used measure of anticoagulation with heparin during extracorporeal circulation. Its routine use in gauging the doses of heparin required in the course of CPB offers distinct advantages over the unmonitored, protocol-directed administration of heparin [150]. Baseline ACT levels, to which post-heparin-reversal levels are compared, must be established after the induction of anesthesia and the opening of the chest, since anesthesia and surgery have been shown to reduce the ACT [151, 152].The optimal level at which the ACT should be kept

during CPB has been debated. In most centers, after the initial heparin dose, the ACT is maintained above 480 seconds by the periodic administration of heparin during CPB.

The anticoagulant effect of heparin is reversed at the termination of CPB by protamine sulfate in incremental doses until the ACT returns to its preheparinization level. Protamine has to be administered slowly because of its frequent adverse hemodynamic effects, including hypotension [153]. Contrary to previous beliefs, the rapid administration of protamine into the aorta has not been shown to be safer than the administration into the central venous system and does not prevent or reduce adverse hemodynamic effects [154, 155].

Although uncommon, the administration of protamine may elicit a severe hemodynamic derangement, characterized by marked pulmonary vasoconstriction, acute pulmonary hypertension, and peripheral vascular collapse, which can be effectively treated with the intravenous administration of PGE_1 [156]. Protamine sulfate has been shown to elicit complement activation by the classic pathway, and a correlation exists between the severity of complement activation and the subsequent hemodynamic derangements [157-160]. High levels of antiprotamine IgE antibody have been identified in the serum of a sensitized patient with protamine-induced fatal anaphylaxis and in the serum of diabetic patients who had received insulin-containing protamine, indicating that the routine administration of protamine to such susceptible individuals is inadvisable [161].

Although the adminsitration of heparin and protamine are guided by the ACT in the vast majority of patients undergoing CPB, it remains uncertain whether monitoring the ACT alone ensures the optimal heparin and protamine doses during and following cardiopulmonary bypass. The correlation between the ACT and the plasma heparin levels is debatable, particularly during the period of CPB. In some studies, ACT levels correlated well with actual heparin levels [162], and provided adequate management during long-term anticoagulation for extracorporeal respiratory assistance [163, 164]. More recent studies, however, have shown a poor correlation between ACT levels and heparin concentrations in the course of cardiopulmonary bypass [165, 166]. This poor correlation is in part due to the effect of hypothermia and hemodilution on the ACT; both of these variables have been shown to prolong the ACT in the course of CPB[165-167].

To ensure more optimal dosing of heparin and protamine in the course of cardiac surgery, a new heparin management system (HMS) has been proposed. The Hepcon/HMS device (Medtronic Blood Management, Parker, CO), which measures both the ACT and whole blood heparin concentration [168], uses heparin/protamine titration [126] to determine quantitatively the targeted heparin concentration for each patient. In the course of the operation, heparin is administered so as to maintain the targeted levels

of both the heparin concentration and the ACT [169, 170]. This more precise manner of instituting, monitoring, and reversing anticoagulation has been shown to improve hemostasis and reduce blood loss and the need for transfusions [169, 171], although the reliability of the Hepcon system in accurately reflecting plasma heparin concentration has been recently questioned [170]. Wang and colleagues [166] have recently proposed a new test, the high-dose thrombin time (HiTT), for monitoring the adequacy of anticoagulation. They showed a direct correlation between HiTT and heparin concentration throughout the period of cardiopulmonary bypass. HiTT was also unaffected by hypothermia and hemodilution [166].

Limitations of systemic anticoagulation with heparin

Although heparin is universally employed for anticoagulation during cardiopulmonary bypass, its ability to achieve optimal and safe anticoagulation is limited by a number of adverse effects. As noted above, heparin, *prior* to the institution of cardiopulmonary bypass, causes significant platelet dysfunction and elicits a fibrinolytic process which, in turn, augments the platelet dysfunction [88, 89]. Since it is unlikely that the administration of protamine completely reverses these adverse effects, the hemostatic dysfunction attributed to cardiopulmonary bypass may, in part, be due to the administration of heparin. Post-CPB mediastinal drainage has been shown to correlate strongly with increased heparin concentration during cardiopulmonary bypass [152].

Heparin also does not completely prevent prothrombin activation and thrombin activity during CPB [32, 152, 172,173]. Despite heparin concentrations adequate to maintain the activated clotting time greater than 400 seconds, prothrombin fragment 1.2 (a byproduct of the cleavage of prothrombin to thrombin), fibrinopeptide A (the first thrombin cleavage product of fibrinogen), thrombin-antithrombin III complex, and fibrin monomer all increase significantly during CPB (Figure 7). Of note is the two-fold increase in prothrombin fragment 1.2, which is observed few hours *after* the termination of CPB and the administration of protamine (Figure 7). This increase in prothrombin fragment 1.2, which may be observed a beyond 24 hours post-operatively, reflects a marked increase in thrombin generation compared to the pre-CPB baseline. As such, it may be indicative of a post-CPB hypercoagulable state which, hypothetically, may contribute to postoperative thrombotic events, such as acute myocardial infarction and cerebrovascular accidents [173].

An increase in the bleeding following CPB may occur secondary to "heparin rebound," which is thought to be due to: (i) an increase in circulating levels of heparin; (ii) increased levels of antithrombin;

or (iii) heparin-protamine complexes formed as a result of excess protamine [174]. A probable frequent cause of heparin rebound is the transfer of cold, heparin-containing extracellular fluid from the periphery into the central circulation, which occurs as a result of rewarming and vasodilation in the postoperative period. Inadequate systemic rewarming prior to termination of CPB predisposes the patient to this phenomenon. Another possible cause for heparin rebound is the transfusion of fresh-frozen plasma, that may provide an increase in antithrombin levels. Heparin may also remain complexed in the presence of excess protamine, but is subsequently liberated as the protamine is metabolized, resulting in increased antithrombin activity. Increased protamine by itself does not have an anticoagulant effect, although when protamine is complexed with heparin, heparin rebound with increased postoperative bleeding may occur [174]. Teoh and colleagues [175] investigated the mechanism of heparin rebound by using chemically modified heparin that lacks anticoagulant activity but that is able to displace protein-bound heparin with anticoagulant activity. Their data suggested that, after administration to patients undergoing cardiac surgery, heparin binds to plasma proteins and is incompletely removed by protamine. After protamine is cleared, the protein-bound heparin dissociates slowly and produces an anticoagulant effect by increasing antithrombin activity [175]. When chemical rather than biologic measurements were made of post-CPB heparin levels in samples from 27 patients undergoing routine coronary revascularization, there was no evidence of persistent heparin in 99.6% of the samples, raising doubts as to the actual concept of heparin rebound [176]. It is now becoming increasingly evident that heparin rebound is unlikely to occur in patients in whom systemic normothermia is maintained during CPB, and in whom the dosing of heparin and protamine is determined more precisely by titration and monitoring using a heparin management system.

The most serious limitation of the use of heparin in cardiac surgery is heparin-induced thrombocytopenia and its sequela of intravascular thrombosis. Heparin-induced thrombocytopenia, which is covered in detail in Chapter 29, is of two types [177]. One type is a transient thrombocytopenia of immediate onset and mild degree and accompanies heparin therapy in approximately 5% of patients [177]. The mechanism is probably nonimmune and related to a direct proaggregatory effect of heparin on platelets [178]. The other type occurs much less frequently in patients receiving heparin and is a delayed, severe, and probably an immune-mediated thrombocytopenia that may occur in association with platelet activation, aggregation, and, on occasion, massive arterial thrombosis [177]. Patients known to be predisposed to heparin-induced thrombocytopenia and thrombosis present a major challenge if they need to be placed on CPB. Management of these patients has included pretreatment with aspirin and dipyridamole [179], pretreatment with iloprost [41], and anticoagulation with low-molecular-weight

heparins and heparinoids [180-184], although these heparin congeners still retain the ability to induce the syndrome. Of particular promise in the treatment of this difficult patient group is the use of ancrod [185,186] or hirudin [187-189] instead of heparin for anticoagulation during CPB.

Heparin-coated extracorporeal circuits

In vitro and animal studies have clearly documented the biocompatibility of oxygenators and tubings coated with endpoint-attached heparin [190-199]. These experimental studies demonstrated that compared to non-coated circuits, heparin-coated surfaces result in reduced complement and platelet activation, increased hematocrit and platelet count, decreased plasma hemoglobin level, improved intraoperative hemodynamics, decreased pulmonary injury, and reduced postoperative blood loss and blood transfusion requirements. The majority of the clinical studies which have compared heparin-coated surfaces to conventional circuits in patients undergoing cardiac surgery [172, 200-211] have demonstrated advantages of the heparin-coated surfaces over the conventional surfaces. These clinical studies fall into one of two categories: 1) Studies in which standard heparin doses were employed in both the heparin-coated and the conventional surface groups, targeting an ACT ≥400-480 seconds [172, 200-205]. and 2) studies in which low-dose heparin was employed in the heparin-coated group, targeting an ACT ≥250 seconds in that group [141, 206-211]. Two important studies in the first category failed to demonstrate a major advantage of the heparin-coated circuits. Gorman and colleagues [172], in a randomized study of 20 patients undergoing myocardial revascularization, demonstrated a significant improvement in platelet function in patients in whom heparin-coated surfaces were employed. However, their data showed no significant differences between groups in fibrinopeptide A, prothrombin fragment F1.2, and thrombin-antithrombin complex concentrations. No significant differences were observed between groups in markers of fibrinolysis and in postoperative clinical outcome parameters, including postoperative blood loss. Muchrcke and colleagues [205], in a randomized study comprising 50 patients who underwent reoperative coronary artery surgery, also failed to demonstrate any significant differences in hematologic parameters and in clinical outcome variables between the two patient groups. The authors of these two studies concluded that heparin-coated cardiopulmonary bypass circuits did not improve biochemical or clinical markers of biocompatibility in their two respective patient populations. Several other clinical studies, in which standard heparin doses were used, provided contrasting data which demonstrated, although not consistently, improved biocompatibility and outcomes in the patient groups subjected to heparin-coated surfaces [200-203].

The main advantage of heparin-coated circuits seems to be realized in the patient groups in which heparin coating is combined with the administration of low-dose heparin and, hence, low-dose protamine. All the studies which included this patient group [141, 206-211] have concluded that low dose heparinization in combination with heparin coating was safe and advantageous. Reduced systemic heparinization (ACT >250 seconds) in patients having extracorporeal circulation with heparin-coated circuits resulted in decreased complement activation [207] and in reduced postoperative morbidity [210], including postoperative blood loss [141, 207, 209, 210], it also did not lead to increased thrombogenicity [208]. Confirmatory studies are needed to ensure that excessive thrombin is not generated with the use of low-dose heparin in combination with heparin-coated circuits.

Alternatives to systemic anticoagulation with heparin

Decreasing the weight-average molecular weight of heparin decreases its antithrombin activity while retaining its anti-Xa activity [180, 212]. Hence, a variety of low-molecular-weight heparins, heparin fractions, and heparinoids are currently being explored as alternatives to unfractionated heparin for anticoagulation during CPB. The ability of these compounds to anticoagulate blood is measured by their anti-Xa activity in units per kilogram body weight. Experimental studies in animals, which included dogs placed on CPB, have demonstrated that these compounds are associated with a lower incidence of hemorrhage than standard heparin at equivalent anti-Xa activity [213-215]. However, low-molecular-weight heparins may produce complications when emergency neutralization procedures are required [216]. When used as anticoagulants for CPB, severe postoperative hemorrhage has been encountered [181, 217]. The use of fractionated low-molecular-weight heparins and heparinoids for anticoagulation during CPB has been restricted to situations in which a standard heparin regimen cannot be used, such as in severe heparin-induced thrombocytopenia and thrombosis [183, 184]. Low molecular weight heparin, however, only reduces the risk of heparin-induced thrombocytopenia and thrombosis; it does not totally eliminate it. *In-vitro* [218] and *in-vivo* studies in the dog [219, 232] and the pig [221] provide evidence that low-molecular weight heparin compounds might be safe and effective enough to be considered as an alternative to heparin in the future.

A number of other compounds are currently being investigated as potential replacements for heparin during cardiac surgery. Hirudin and ancrod are two pharmacologic agents that have been used clinically, mostly in patients known to have heparin-induced thrombocytopenia. Hirudin is an antithrombin-independent thrombin inhibitor which, unlike heparin, can inactivate thrombin bound to

fibrin [222-224]. Recombinant hirudin has been used effectively as an anticoagulant during CPB in experimental animals and in humans [187, 189, 219]. Recent experimental studies in the rat [225] have raised concerns about an increased hemorrhagic effect of hirudin compared to other new anticoagulants. A recent clinical study has demonstrated persistent thrombin generation in humans, indicated by increased levels of prothrombin fragment 1.2, during specific thrombin inhibition with hirudin [226]. Hence, although the specificity of hirudin and hirudin congeners (e.g., bivalirudin or Hirulog) for thrombin, and their ability to penetrate formed clots, make them very promising heparin substitutes, more studies are needed before their routine use in cardiac surgery can be advocated.

Ancrod is a defibrinogenating enzyme that has been demonstrated in initial animal and human studies to be safe and effective as a substitute to heparin during CPB [227, 228]. The activated clotting time is not reliable in monitoring the adequacy of anticoagulation with ancrod; measurement of fibrinogen concentration before, during, and following CPB may be the only useful method the anticoagulant adequacy of ancrod [186]. During cardiopulmonary bypass, ancrod is administered as a continuous infusion, and is titrated to achieve plasma fibrinogen concentrations of 0.2-0.7 g/l, however; 12-24 hours of therapy as required to achieve adequate reductions in clottable fibrinogen, thereby limiting its use in elective procedures. Like hirudin, the clinical use of ancrod should be limited to patients in whom anticoagulation with heparin is contraindicated.

Experimental studies have been conducted in canine and porcine models to explore the potential of newer agents as possible alternatives to heparin anticoagulation during CPB. Some of the agents that have been found to be promising in these studies include dermatan sulfate [229], a short-acting oligonucleotide-based thrombin inhibitor (thrombin aptamer) [230], DuP 714, a synthetic peptide thrombin inhibitor [231], and CGP 39393, a specific peptide inhibitor of thrombin [232]. Experimental studies also have demonstrated the feasibility of interposing in the bypass circuit an immobilized heparinized reactor filter [233] and an immobilized protamine bioreactor filter [234, 235], which would eliminate heparin from the blood returned to the patient and reduce both heparin anticoagulant activity and the need for systemic protamine (with its potential adverse reactions). This technology has not yet been applied clinically.

A promising adjunct to systemic heparinization is the use of inhibitors of GPIIb/IIIa receptors. Hiramatsu and his collaborators have demonstrated an impressive preservation of platelet function and complete prevention of platelet loss in pigs subjected to CPB and treated with high dose tirofiban, a nonpeptide inhibitor of platelet GPIIb/IIIa receptors [236]. This novel approach to the reduction of CPB-induced hemostatic dysfunction awaits confirmation in the clinical setting.

In preliminary studies conducted in baboons and humans, recombinant platelet factor 4 has been identified as a possible substitute for protamine in the the reversal of heparin-induced anticoagulation [237-239]. Data from these studies support future clinical trial of this endogenous antiheparin protein.

Non-surgical blood loss during and following cardiopulmonary bypass

Types and measurement of post-CPB blood loss

Clinically, the most important adverse effect of the hemostatic dysfunction observed in patients undergoing cardiac surgery is increased intra- and postoperative blood loss. Increased blood loss following the institution of CPB may be loosely defined as "surgical" or "nonsurgical" in nature. Blood loss from a specific anatomic site as a result of the surgical procedure itself is referred to as "surgical", while diffuse blood loss not associated with a specific anatomic site and which cannot be corrected surgically is referred to as "nonsurgical." The magnitude of postoperative non-surgical blood loss can be reflective of the magnitude of the hemostatic derangement observed in patients undergoing open heart surgery and can be used as an endpoint for the evaluation of the efficacy of interventions designed to reduce the CPB-induced hemostatic defect. However, for postoperative blood loss to reflect the magnitude of hemostatic dysfunction accurately, it has to be properly quantified by observing the following two guidelines: 1) Patients in whom postoperative bleeding is considered to be surgical in nature should be excluded from the analysis; e.g. the patient who is returned to the operating room for repair of an obvious leak in the aortotomy suture line. 2) Measurement of post-CPB blood loss should not only include the postoperative chest tube drainage in the intensive care unit, but also the blood loss encountered in the operating room after the administration of protamine and the normalization of the ACT; i.e., before the chest is closed and the patient is transferred to the intensive care unit. Quantification of this intraoperative portion of the post-CPB blood loss should include measurement of the volume of blood aspirated into the wall suction and/or the Cell Saver during this period, as well as the volume of blood collected into mediastinal and chest tubes after their insertion. In addition, all sponges and laparotomy pads used during this period should be collected separately, weighed, and their blood contents recorded and added to the total blood loss. The weight differential of the sponges and laparotomy pads can be transformed to ml of blood loss using the following formula:

$$\text{ml of blood loss} = \text{Weight in grams} / \text{Blood density}$$

where: Blood density (in grams) = 0.0692 x Hematocrit + 1.0239

0.0692 = Density of cells - density of plasma

1.0239 = Density of plasma

Figure 8 shows the components of post-CPB blood loss measured prospectively by a dedicated research assistant in 172 patients undergoing CPB. In this patient population, the intraoperative component of the blood loss averaged 24.4% of the total blood loss, ranging from 2.4 to 69.9 % of the total blood loss. Ignoring this intraoperative component and restricting the measurement of the blood loss to the chest tube drainage in the intensive care unit can jeopardize the validity of the blood loss measurements. The intraoperative component of the post-CPB blood loss is important in the assessment of the magnitude of the CPB-induced hemostatic dysfunction because surgeons normally would not close the chest and transfer the patient to the intensive care unit until they achieve maximal control of the bleeding in the operative field. Thus, the blood loss incurred during this period might provide more useful information about the tendency of the patient to bleed than does the blood loss incurred in the intensive care unit.

Determinants of post-CPB blood loss

Surgical bleeding during and following CPB is determined primarily by the expertise of the surgical team and the adequacy of the surgical techniques employed. A consensus in the current literature about the determinants of non-surgical bleeding is difficult to achieve because of the wide disparity in the definitions, methods of measurement of non-surgical blood loss, and laboratory analytical techniques. In a prospective study of 100 patients in which surgical bleeders were excluded and which was specifically designed to identify the determinants of the first four hours of post-CPB blood loss (starting with the neutralization of protamine), the following variables were noted to be significant in the univariate analyses (Figure 9): the duration of CPB; the lowest esophageal temperature; the platelet mass during cardiopulmonary bypass; the wound temperature; the hematocrit; the platelet count; and the bleeding time two hours post-CPB. In the multivariate analysis, the two independent variables that were predictive of the blood loss were the duration of CPB and the hematocrit two hours post-CPB [18]. Many other studies have confirmed that the duration of CPB [240, 241], hypothermia [241, 242], and the heparin concentration during CPB [207, 209] are all important predictors of post-CPB blood loss. The finding that hemodilution is a significant independent predictor of blood loss is important; it underscores the potential of red blood cell transfusion as a means to reduce post-CPB blood loss.

Although derangements in a variety of laboratory measurements of blood coagulation and platelet function are observed during and following cardiopulmonary bypass [243, 244], the role of these measurements in predicting post-CPB blood loss and the need for blood product transfusions are controversial. In particular, the role of the bleeding time in the prediction of post-CPB blood loss remains to be defined fully, (also see Chapter 23). It is well established that the preoperative bleeding time, under normal circumstances, does not predict the amount of postoperative blood loss [18, 50, 72, 245-252]. Marked extensions in the preoperative bleeding time, however, have tended to result in increased postoperative blood loss [245]. It has not yet been established whether prolonged preoperative bleeding times in patients receiving preoperative aspirin are predictive of excessive bleeding post-CPB. There are data to suggest that preoperative aspirin ingestion prolongs the preoperative bleeding time but does not influence post-CPB bleeding time [253]. Conversely, a blinded randomized, placebo-controlled Veterans Administration Cooperative Study has demonstrated a significant increase in post-CPB blood loss in patients receiving preoperative aspirin [254]. Unfortunately, no bleeding time measurements were obtained in this study. The validity of published observations related to this issue is limited by: (i) the recent demonstration of the temperature dependence of the bleeding time measurement and the need to correct the bleeding time for temperature [20, 90, 255], and (ii) the failure in most of the published studies to differentiate and quantify properly and prospectively nonsurgical blood loss. In studies in which the bleeding time and the post-CPB blood loss were measured carefully, the *postoperative* bleeding time correlated with the magnitude of the postoperative blood loss, providing a rationale for platelet transfusions in patients with excessive postoperative bleeding and a significant prolongation of the bleeding time [18, 256, 257].

Some studies have identified a role for pre-[240] and postoperative [241, 258] measurements of PT and aPTT in the prediction and clinical management of post-CPB blood loss, (also see Chapter 23). However, the majority of recent studies have failed to associate the pre- and post-CPB PT and aPTT with the magnitude of the post-CPB blood loss [18, 243, 244, 259]. As stated by Brandt [257], the consensus of recent studies addressing the merits of laboratory tests of coagulation, fibrinolysis, and platelet function in patients undergoing CPB conveys two important messages: first, laboratory tests cannot be used to identify patients who are likely to bleed; and, second, there is little indication for an extensive battery of coagulation tests to be conducted routinely following CPB [257].

Prevention and Management of post-CPB blood loss

Preoperative work-up and surgical technique: Performing an adequate preoperative workup

of the cardiac surgical patient is important for the prevention and management of post-CPB blood loss. The preoperative workup should include a thorough history to assess the patient's bleeding tendency and related family history. Preoperative ingestion of aspirin might increase post-CPB bleeding [253] but need not deter the cardiac operation. Preoperatively, it is reasonable to obtain a partial thromboplastin time, prothrombin time, platelet count, and a template bleeding time [260]. In view of the limitations of these tests in the prediction of postoperative blood loss, an alternative approach would be to perform them only in patients with increased risk of bleeding. Protocols have been recently developed to identify such patients preoperatively [261]. In patients with preoperative anemia, the administration of recombinant human erythropoietin has been shown to be useful in reducing postoperative blood loss [262-266].

Probably the single most important factor in the prevention of excessive post-CPB blood loss is meticulous attention to surgical technique intraoperatively. Performing an effective operation expeditiously, reducing the period of CPB to a minimum, and applying proper hemostatic techniques throughout all the phases of the operation are imperative for minimizing post-CPB blood loss.

Extracorporeal perfusion techniques: The conduct and conditions of extracorporeal perfusion significantly influence the genesis and the management of post-CPB blood loss. Adequate systemic rewarming and maintenance of systemic normothermia are very important measures in the prevention and treatment of excessive postoperative blood loss. The rationale for this approach has been elucidated above, and is based on the observed relationships between temperature and post-CPB platelet function and blood loss. Since the hematocrit is an important predictor of post-CPB blood loss, minimizing the degree of hemodilution in the course of cardiac surgery is likely to improve postoperative hemostatic function. Raising the hematocrit during cardiopulmonary bypass can be achieved by a combination of retrograde autologous priming (i.e. replacing the crystalloid prime with blood drained from the right side of the heart via the venous cannula immediately before the institution of CPB [267]), interposition of a hemoconcentrator in the bypass circuit, and red blood cell transfusion.

Reducing the blood-gas interface by using a closed perfusion circuit and by minimizing negative pressure aspiration of blood from the surgical field into the extracorporeal circuit. are also measures that can reduce hemostatic dysfunction and post-CPB blood loss [268]. In addition, blood accumulating in the pericardial cavity and aspirated into the extracorporeal circuit has been shown to cause increased thrombin generation [129] and increased fibrinolysis [130]. Hence, during CPB, aspiration of blood accumulating in the pericardial cavity into the Cell Saver instead of the extracorporeal circuit should reduce the hemostatic dysfunction and decrease postoperative blood loss. Cell Saver blood is washed

before it is retransfused at the end of CPB, ridding it of prothrombotic and profibrinolytic substances.

As discussed above, reducing the ACT from 480 to 250 seconds by the use of heparin-coated circuits has been shown to be safe and is likely to reduce the heparin-induced hemostatic dysfunction and, hence, post-CPB blood loss.

Autologous and homologous blood product transfusions: Transfusion of miscellaneous blood products should be administered following significant blood loss. Autologous blood, collected over a period of 3-4 weeks preoperatively, is the optimal replacement, although it may not be logistically feasible in a number of patients [269-271]. Phlebotomy of whole blood immediately prior to the institution of CPB and its subsequent postoperative reinfusion has not been shown to be consistently beneficial [272-276]. The routine use of homologous platelet transfusions in cardiac surgery has been determined to be totally unnecessary [277]. Newer methods and techniques have made possible the sequestration of platelet-rich plasma (with retransfusion of the red cells) immediately prior to institution of CPB and reinfusion of this plasma postoperatively after discontinuation of bypass. This technique spares the platelets, in part, from being affected by the extracorporeal circuit. Preservation of platelet number, a decrease in the postoperative blood loss, and a decrease in the requirement for homologous blood transfusions have been reported with this technique [278-281]. Cell Savers are routinely used in the course of open-heart surgery, and washed red cells are autotransfused after completion of CPB. Blood shed from the pleura and mediastinum through the chest tubes during the first 24 hours is reinfused in an effort to reduce the need for postoperative homologous blood transfusion [272-285]. Transfusion of up to 1 liter of unwashed shed mediastinal blood is safe, and except for its possible effect on the hematocrit, does not effect on any of the hematologic or related parameters of the recipient patient [286]. The safety of larger transfusions of unwashed, shed mediastinal blood has yet to be determined.

The treatment of excessive postoperative nonsurgical bleeding usually entails the transfusion of platelets (see Chapter 51), cryoprecipitate, and fresh frozen plasma (see Chapter 52). These products should not be transfused before (I) heparin is adequately reversed, (ii) full systemic normothermia is achieved, and (iii) the hematocrit is restored to a value exceeding 30% with red blood cell transfusions. Observing these three conditions should reduce the need for fresh frozen plasma and platelet transfusions postoperatively. Although current storage techniques allow for platelets to be transfused up to five days after pheresis, platelet function progressively deteriorates during liquid storage. Actively bleeding patients who lack adequate numbers of functional platelets should receive platelets that have been stored at room temperature for no more than 24 hours [288]. Preliminary clinical data indicate that infusion of thawed cryopreserved platelets (stored at -80°C improves the function of platelets stored for an average

of 289 ±193, median=250 days, range of 30-720 days), compared to liquid-stored platelets, significantly reduces postoperative blood loss and the need for blood product transfusions [289].

Pharmacologic interventions: Various pharmacologic agents have been used to reduce bleeding after CPB. PGE_1 and PGI_2 have been shown to reduce platelet loss in *in vitro* simulation of extracorporeal circulation [37, 38, 289]. However, randomized, double-blind, controlled trials of prophylactic PGI_2 administration in patients undergoing CPB have not shown clear evidence of benefit [41, 42, 67, 68, 289-292]. Furthermore, PGI_2 and its analog iloprost cause severe vasodilation and hypotension [41, 42, 67, 68, 289]. Both dog [43] and human [44] studies have also shown that the preoperative administration of dipyridamole preserves the platelet count and reduces blood loss following cardiac surgery. The routine clinical use of these pharmacologic agents, however, has not been established [68].

It is now clearly established that antifibrinolytic agents (Chapter 53) have a significant role in reducing CPB-induced hemostatic dysfunction and post-CPB blood loss. The salutary role of these agents confirms the important role hyperfibrinolysis plays in the genesis of hemostatic dysfunction during cardiac surgery. ϵ-amino caproic acid (Amicar) was originally used in selected patients and proved to be effective in controlling excessive postoperative blood loss following CPB [119, 125, 293]. The prophylactic intravenous administration of ϵ-aminocaproic acid (10 gm before skin incision, 10 gm after heparin administration, and 10 gm at the discontinuation of CPB; or 10 gm at induction of anesthesia followed by an infusion of 2 gm/hour for 5 hours) has been recently shown to be safe and to reduce fibrinolysis and blood loss after cardiac surgery significantly [94, 294]. Its prophylactic administration in a single dose of 5 gm prior to the institution of CPB has also been shown to reduce blood loss when combined with the postoperative administration of desmopressin [295]. Tranexamic acid is an isomer of ϵ-aminocaproic acid, with 7-10 times its inhibitory activity [296]. Several recent studies have also shown it to be effective in reducing blood loss when administered prophylactically to patients undergoing cardiac surgery [297-301]. Tranexamic acid may be less effective than ϵ-amino-caproic acid in the prevention of post-CPB blood loss [294]; its prophylactic administration to children seems to be effective only in patients with cyanotic heart disease [302]

Aprotinin, a protease inhibitor, has had a consistent record of significantly reducing postoperative blood loss: reductions of 40-60% were observed when this pharmacologic agent was administered in high doses before and throughout the period of CPB [116, 303-308]. Recent studies have indicated that low dose aprotinin (50-80% the high dose), when administered before and during CPB, is also effective in reducing the hemostatic dysfunction and the postoperative blood loss in patients

undergoing all types of cardiac surgery [308-313]. It is now becoming increasingly evident that aprotinin, particularly when administered in a low dose, exerts its salutary effect mainly by inhibiting hyperfibrinolysis [116, 307, 311-315]. Whether aprotinin has a protective effect on the platelet during CPB remains controversial. Aprotinin administration has been reported to improve platelet function [91, 308,316] and ultrastructure [309]. However, in a well conducted double-blind study, convincing data were provided which indicated that aprotinin did not influence the change in platelet count, did not suppress beta-thromboglobulin release from platelets, did not prevent the CPB-induced inhibition of platelet function, and did not influence the concentration of plasma glycocalicin during and following CPB [314]. These results have recently been confirmed by others [317]. A recent double-blind randomized study of 106 patients undergoing CPB also concluded that the mechanism by which high dose aprotinin reduced bleeding was independent of any effect on platelet function. This study did demonstrate, however, that aprotinin produced a greater reduction in bleeding among patients whose condition was hemostatically compromised by preoperative platelet dysfunction (318). The administration of aprotinin in the presence of heparin causes a prolongation of the ACT and the aPTT. The prolongation of the ACT occurs only when celite-activated tubes are used in the measurement of the ACT; it is not observed when kaolin-activated tubes are used [319]. This has prompted the use of kaolin instead of celite as the activating agent for measuring the ACT in patients in whom aprotinin is used. A recent study has ascribed the extension of the celite-based ACT to an anitcoagulant effect of aprotinin [306] and has advocated the use of celite rather than kaolin as the activating factor in these patients. It is now becoming increasingly evident that both methods of ACT monitoring are equally reliable in following anticoagulation in patients receiving aprotinin, particulary in conjunction with the Hepcon/Heparin Management System [320, 321]. Thrombin-based tests, such as the high-dose thrombin time (HITT), have also been used to monitor the adequacy of anticoagulation in these patients [322]. The prolongation of the celite-based ACT and the aPTT with the administration of aprotinin has led some investigators to hypothesize that a synergistic relationship exists between aprotinin and heparin, and to advocate reducing the usual heparin dose in patients receiving aprotinin during CPB [323]. Concern had been expressed about the possibility of aprotinin causing a hypercoagulable state postoperatively, and resulting in an increased incidence of postoperative aortocoronary graft occlusion. These concerns have been alleviated by several studies that have demonstrated *decreased* thrombin generation postoperatively in adults and children receiving high-dose aprotinin [306, 307, 315] and by a placebo-controlled, double-blind study conducted in 90 patients which showed that early (7-12 day) vein graft patency was not adversely affected by high-dose aprotinin [324]. A recent study examined the *in vitro* vascular reactivity

of coronary bypass grafts to a range of vasoconstrictor agents in the presence or absence of aprotinin. Aprotinin resulted in the preservation of endothelium dependent responses to acetylcholine and in a decrease in the vasoconstrictive response to the thromboxane analogue U46619. Hence, it was postulated that the decrease in vasoconstriction following the administration of aprotinin could counteract potential prothrombotic adverse effects on graft patency [325]. Renal dysfunction and intravascular coagulation have been reported following the administration of aprotinin to elderly patients undergoing operations on the thoracic and thoracoabdominal aorta using cardiopulmonary bypass and hypothermic circulatory arrest [326]. As an allogenic protein, aprotinin possesses antigenic properties. The incidence of adverse reactions to re-exposure to aprotinin has been recently reported by Dietrich et al [327] to be 2.8%. The severity of the reactions to aprotinin in this series varied over a wide spectrum but did not result in any fatality. The authors recommended the following procedure for re-exposure with high-dose aprotinin: (1) delay of the first bolus injection of aprotinin until the surgeon is ready to begin CPB, (2) test dose of 10,000 KIU aprotinin in all patients with aprotinin treatment, (3) H_1/H_2 blockade in known or possible reexposures, and)4) avoidance of reexposure within the first 6 months after the previous exposure to aprotinin [327]. Recent comparative studies have shown aprotinin to be equally effective in reducing blood loss as ϵ-amino-caproic acid [294], but more effective than tranexamic acid [294, 328].

Intravenous DDAVP (see Chapter 53) has been used to reduce post-CPB blood loss because of its potential to raise the plasma levels of vWF and its multimers. However, inconsistent and contradictory results have been reported with the use of this drug in the postoperative period [73, 329-324]. The original report by Salzman and colleagues. [329] showed DDAVP to be beneficial in increasing the vWF level and in reducing the postoperative blood loss in patients undergoing complex valvular heart surgery. Subsequent studies in patients undergoing routine coronary artery revascularization [73] and in children undergoing cardiac operations [332] showed that the administration of DDAVP did not increase the levels of vWF over and above the rise ordinarily observed postoperatively in the cardiac patient, nor did it reduce postoperative blood loss. These latter results, along with the finding that the level of vWF in a large group of patients undergoing coronary and valvular operations did not correlate with post-CPB blood loss [18], indicate that DDAVP should not be used as a routine adjunct in cardiac surgery. It might be useful in patients known to have platelet dysfunction preoperatively [333, 334], or in combination with ϵ-amino-caproic [295].

Summary

The institution of CPB elicits hemostatic abnormalities that result in increased postoperative blood loss. The two major CPB-induced hemostatic abnormalities are platelet dysfunction and hyperfibrinolysis.

The prolongation of the bleeding time during and following CPB and its reversal within 24 to 48 hours postoperative are indicative of CPB-induced reversible platelet dysfunction. Thrombocytopenia, which is observed during and following CPB, results from hemodilution and from the loss of platelets, which are activated and degranulated through contact with and adhesion to synthetic surfaces. The degree of thrombocytopenia observed during CPB is not severe enough to account for the prolongation of the bleeding time and for the platelet dysfunction observed. Recent studies employing whole blood flow cytometry have shown that CPB results in: 1) markedly deficient platelet reactivity in response to an *in vivo* wound, 2) normal platelet reactivity *in vitro*, 3) no loss of the platelet surface GPIb-IX or GPIIb-IIIa complexes, and 4) a minimal number of circulating activated platelets. These studies suggest that the "platelet function defect" of CPB is not a defect intrinsic to the platelet, but is mostly caused by a variety of extrinsic factors some of which are yet to be identified. Systemic hypothermia, fibrinolysis, and heparin are extrinsic factors that have been shown to contribute to the platelet dysfunction observed during and following CPB.

The administration of heparin, the institution of CPB, and blood stasis in the pericardial cavity result in increased fibrinolysis, which hinders post-CPB hemostasis. The prophylactic use of antifibrinolytic agents, in particular aprotinin and ϵ-aminocaproic acid, has been shown to reduce blood loss following CPB effectively.

When properly measured, nonsurgical blood loss can be reflective of the magnitude of the hemostatic abnormality observed following the institution of CPB. The quantification of this blood loss should begin intraoperatively, immediately after the neutralization of heparin and the normalization of the ACT. Nonsurgical post-CPB blood loss is determined primarily by the duration of CPB and by a number of other factors, which include the level of hemodilution, the systemic and skin temperatures, the heparin concentration (and the adequacy of its reversal), and the platelet mass during and following CPB. Routine tests of coagulation have not been shown to be consistently predictive of the post-CPB blood loss. The preoperative bleeding time is not predictive of the postoperative blood loss, but the bleeding time during the initial postoperative period is predictive of the blood loss during that period.

Clinical measures with a potential for limiting the hemostatic defect and for reducing the post-CPB blood loss include the maintenance of systemic normothermia, the complete reversal of systemic hypothermia, minimization of hemodilution, the employment of a closed perfusion circuit, avoidance of excessive aspiration of blood into the pump, avoidance of blood stasis in the pericardial cavity, and the

accurate monitoring of anticoagulation by the use of a heparin management system. Heparin remains the universal anticoagulant for CPB. However, heparin has numerous adverse effects and its use is limited in conditions of heparin-induced thrombocytopenia and thrombosis.

Heparin, in its current clinical dose, does not completely prevent thrombin generation during and, more importantly, *following* the institution of CPB. Alternatives to heparin have been employed; their use continues to be sporadic and limited to patients who cannot receive heparin. The use of heparin-coated surfaces may enable the reduction of the heparin dose employed during CPB. The safety and efficacy of low-dose heparin with heparin-coated surfaces is currently under intense investigation.

References

1. Roth GJ. Developing relationships: arterial platelet adhesion, glycoprotein Ib, and leucine-rich glycoproteins. Blood 1991;77:5-19.

2. Michelson AD, Loscalzo J, Melnick B, et al. Partial characterization of a binding site for von Willebrand factor on glycocalicin. Blood 1986;67:19-26.

3. Phillips DR, Charo IF, Parise LV, et al. The platelet membrane glycoprotein IIb-IIIa complex. Blood 1988;71:831-843.

4. Leung LLK. The role of thrombospondin in platelet aggregation. J Clin Invest 1984;74:1764-1772.

5. Bevilacqua M, Butcher E, Furie B, et al. Selectins: A family of adhesion receptors (letter). Cell 1991 Oct 18;67(2):233.

6. McEver RP. Properties of GMP-140, an inducible granule membrane protein of platelets and endothelium. Blood Cells 1990;16(1):73-80,discussion 80-83.

7. Hsu-Lin SC, Berman CL, Furie BC, et al. A platelet membrane protein expressed during platelet activation and secretion. Studies using a monoclonal antibody specific for thrombin-activated platelets. J Biol Chem 1984;259:9121-9126.

8. Stenberg PE, McEver RP, Shuman MA, et al. A platelet alpha-granule membrane protein (CPM-140) is expressed on the plasma membrane after activation. J Cell Biol 1985;101:880-886.

9. Larsen, E, Celi A, Gilbert GE, Furie BC, et al. PADGEM protein: A receptor that mediates the interaction of activated platelets with neutrophils and monocytes. Cell 1989;59:305-312.

10. Hamburger SA and McEver RP. GMP-140 mediates adhesion of stimulated platelets to neutrophils. Blood 1990;75:550-554.

11. Palabricia T, Lobb R, Furie BC, et al. Leukocyte accumulation promoting fibrin deposition is mediated in vivo by P-selectin on adherent platelets. Nature 1992 359:848-851.

12. Celi A, Pellegini G, Lorenzet R, et al. P-selectin induces the expression of tissue factor on monocytes. Proc. Natl. Acad. Sci. U.S.A. 1994;91:8767-8771.

13. George JN, Pickett EB, Saucerman S, et al. Platelet surface glycoproteins. Studies on resting and activated platelets and platelet membrane microparticles in normal subjects, and observations in patients during adult respiratory distress syndrome and cardiac surgery. J Clin Invest 1986;78:340-348.

14. Michelson AD, Barnard MR. Thrombin-induced changes in platelet membrane glycoproteins Ib, IX, and IIb-IIIa complex. Blood 1987;70:1673-1678.

15. George JN, Torres MM. Thrombin decreases von Willebrand factor binding to platelet glycoprotein Ib. Blood 1988; 71:1253-1259.

16. Michelson AD, Ellis PA, Barnard MR, et al. Downregulation of the platelet surface glycoprotein Ib-IX complex in whole blood stimulated by thrombin, ADP or an in vivo wound. Blood 1991;77:770-779.

17. Harker LA, Malpass TW, Branson HE,et al. Mechanism of abnormal bleeding in patients undergoing cardiopulmonary bypass: acquired transient platelet dysfunction associated with selective α-granule release. Blood 1980;56:824-834.

18. Khuri SF, Wolfe JA, Josa M, et al. Hematologic changes during and following cardiopulmonary bypass and their relationship to the bleeding time and non-surgical blood loss. J Thorac Cardiovasc Surg 1992;7:94-107.

19. Kestin AS, Valeri CR, Khuri SF, et al. The platelet function defect of cardiopulmonary bypass. Blood 1993;82:107-117.

20. Valeri CR, Khabbaz K, Khuri SF, et al. Effect of skin temperature on platelet function in

extracorporeal bypass patients. J Thorac Cardiovasc Surg 1992;7:108-116.

21. Edmunds, LH, Addonizio VP. Extracorporeal circulation. In:Colman RW, Hirsh J, Marder VJ, Salzman EW, eds. Hemostasis and thrombosis. Basic principles and clinical practice. Philadelphia: JB Lippincott, 1987:901-912.

22. Mammen EF, Kooets MH, Washington BC, et al. 1985. Hemostasis changes during cardiopulmonary bypass surgery. Semin Thromb Hemost 1985;11:281-292.

23. Colman RW. Platelet and neutrophil activation in cardiopulmonary bypass. Ann Thorac Surg 1990;39:32-34.

24. Martin JF, Daniel TD, Trowbridge EA. Acute and chronic changes in platelet volume and count after cardiopulmonary bypass induced thrombocytopenia in man. Thromb. Haemostas. 1987;57:55-58.

25. Addonizio VP, Colman RW, Edmunds LH. Effect of blood flow and surface area on platelets during extracorporeal circulation. Trans Am Soc Artif Intern Organs 1978;24:650-655.

26. Salzman EW, Linden J, Brier D, et al. Surface-induced platelet adhesion, aggregation and release. Ann. NY Acad. Sci.1977;283:114-127.

27. George JN. Direct assessment of platelet adhesion to glass: A study of the forces of interaction and the effects of plasma and serum factors, platelet function and modification of the glass surface. Blood 1982;40:862-874.

28. Uniyal S, Brash JL. Patterns of adsorption of proteins from human plasma onto foreign surfaces. Thromb Haemostas 1982;47:285-290.

29. Lindon JN, McNamara G, Kushner L, et al. Does the conformation of adsorbed fibrinogen dictate platelet interactions with artificial surfaces? Blood 1986;68:355-362.

30. Lindon J, McNamara G, Pekala R, et al. Fibrinogen platelet interaction on hydrophilic and

hydrophobic surfaces. Circulation 1984;70:II358-II364.

31. Glusko R, Rucinski B, Musial J, et al. Fibrinogen receptors in platelet adhesions to surfaces of extracorporeal circuit. Am J Physiol 1987;21:H615-H621.

32. Slaughter TF, LeBleu TH, Douglas JM Jr, et al. Characterization of prothrombin activation during cardiac surgery by hemostatic molecular markers. Anesthesiology 1994;80(3):520-526.

33. Chuang HYK, Sharma NC, Mohammed SF, et al. Adsorption of thrombin onto artificial surfaces and its detection by an immunoradiometric assay. Artif Organs 1979;3:226-231.

34. Shattil SJ, Hoxie JA, Cunningham M, et al. Changes in the platelet membrane glycoprotein IIb-IIIa complex during platelet activation. J Biol Chem 1985;260:11107-11114.

35. Musial J, Niewiarowski S, Hershock D, et al. Loss of fibrinogen receptors from the platelet surface during extracorporeal circulation. J Lab Clin Med 1985;105:514-522.

36. Teoh KH, Christakis GT, Weisel RD, et al. Prevention of myocardial platelet deposition and thromboxane release with dipyridamole. Circulation 1986;74:III145-III152.

37. Addonizio VP Jr, Macarak EJ, Nicolau KC, et al. Effects of prostacyclin and albumin on platelet loss during in vitro stimulation of extracorporeal circulation. Blood 1979;53:1033-1042.

38. Addonizio VP, Maracak EJ, Niewiarowski S, et al. Preservation of human platelets with prostaglandin E_1 during in vitro simulation of cardiopulmonary bypass. Circ Res 1979,44:350-357.

39. Addonizio VP, Fisher CA, Jenkin BK, et al. Iloprost (ZK36374), a stable analogue of prostacyclin, preserves platelets during simulated extracorporeal circulation. J Thorac Cardiovasc Surg 1985;89:926-933.

40. Addonizio VP Jr, Fisher CA, Kappa JR, et al. Prevention of heparin-induced thrombocytopenia during open heart surgery with iloprost (ZK36374). Surgery 1987 Nov;102(5):796-807.

41. Walker ID, Davidson JF, Faichney A, et al. A double blind study of prostacyclin in cardiopulmonary bypass surgery. Br J Haematol 1981;49:415-423.

42. Aren C, Feddersen K, Radegran K. Effects of prostacyclin infusion on platelet activation and postoperative blood loss in coronary bypass. Ann Thorac Surg 1983;36:49-54.

43. Teoh KH, Christakis GT, Weisel RD, et al. Dipyridamole reduced myocardial platelet and leukocyte deposition following ischemia and cardioplegia.
J Surg Res 1987;42(6):642-652.

44. Teoh KH, Christakis GT, Weisel RD, et al. Dipyridamole preserved platelets and reduced blood loss after cardiopulmonary bypass. J Thorac Cardiovasc Surg 1988;96(2):332-341.

45. Packham MA, Evans G, Glynn MF, et al. The effect of plasma proteins on the interaction of platelets with glass surfaces. J Lab Clin Med 1969;73:686-697.

46. Salzman EW, Merrill EW, Binder A, et al. Protein-platelet interaction on heparinized surfaces. J Biomed Mater Res 1969;3:69-75.

47. Edmunds LH, Saxena NC, Hillyer P, et al. Relationship between platelet count and cardiotomy suction return Ann Thorac Surg 1978;25:306-310.

48. Boonstra PW, van Imhoff GW, Eysman L, et al. Reduced platelet activation and improved hemostasis after controlled cardiotomy suction during clinical membrane oxygenator perfusion. J Thorac Cardiovasc Surg 1985;89:900-906.

49. Edmunds LH, Jr., Ellison N, Colman RW, et al. Platelet function during open-heart surgery: Comparison of membrane and bubble oxygenators. J Thorac Cardiovasc Surg 1982;83:805-812.

50. Bick RL. Hemostasis defects associated with cardiac surgery, prosthetic devices, and other extracorporeal circuits. Semin Thromb Hemost 1985;11(3):249-280.

51. Bachmann F, McKenna R, Cole ER, et al. The haemostatic mechanism after open-heart surgery. J Thorac Cardiovasc Surg 1975;70:76-85.

52. Young JA. Coagulation abnormalities with cardiopulmonary bypass. In: Utley JR, ed. Pathophysiology and techniques of cardiopulmonary bypass. Baltimore: Williams and Wilkins, 1982;88-105.

53. Ekert H, Gilchrist GS, Stanton R, et al. Hemostasis in cyanotic congenital heart disease. J Pediatr 1970;76:221-230.

54. Gross S, Keefer V, Liebman J. The platelet in cyanotic congenital heart disease. Pediatrics 1968;42:651-655.

55. Laufer N, Merin G, Grover NB, et al. The influence of cardiopulmonary bypass on the size of human platelets. J Thorac Cardiovasc Surg 1975; 70:727-731.

56. Thompson CB, Jakubowski JA, Quinn PG, et al. Platelet size as a determinant of platelet function. J Lab Clin Med 1983;101:205-213.

57. Davey FR, Parker FB. Delayed hemostatic changes following cardiopulmonary bypass. Amer J Med Sci 1976;271:171-178.

58. Cella G, Vittadello O, Gallucci V, et al. The release of β-thromboglobulin and platelet factor 4 during extracorporeal circulation for open heart surgery. Europ J Clin Invest 1981;11:165-169.

59. Addonizio VP Jr, Smith JB, Strauss JF 3rd, et al. Thromboxane synthesis and platelet secretion during cardiopulmonary bypass with bubble oxygenator. J Thorac Cardiovasc Surg 1980;79:91-96.

60. Mezzano D, Aranda E, Urzua J, et al. Changes in platelet beta-thromboglobulin, fibrinogen, albumin, 5-hydroxytryptamine, ATP and ADP during and after surgery with extracorporeal circulation in man. Am J Hematol 1986;22:133-142.

61. Pumphrey CW, Dawes J. Platelet alpha granule depletion: findings in patients with prosthetic heart valves and following cardiopulmonary bypass surgery. Thromb Res 1983;30:257-264.

62 Zilla P, Fasol R, Groscurth P, et al. Blood platelets in cardiopulmonary bypass operations. Recovery occurs after initial stimulation, rather than continual activation. J Thorac Cardiovasc Surg 1989;97:379-388.

63. Valeri CR, Feingold H, Cassidy G, et al. Hypothermia-induced reversible platelet dysfunction. Ann Surg 1987;205:175-181.

64. Thorngren M, Shafi S, Born GV. Thromboxane A2 in skin-bleeding-time blood and in clotted venous blood before and after administration of acetylsalicylic acid. Lancet 1983;1:1075-1078.

65. Gerrard JM, Taback S, Singhroy S, et al. In vivo measurement of thromboxane B2 and 6-keto-prostaglandin F1a in humans in response to a standardized vascular injury and the influence of aspirin. Circ. 1989;79:29-38.

66. Hennessy VL, Hicks RE, Niewiarowski S, et al. Function of human platelets during extracorporeal circulation. Am J Physiol 1977;232:H622-H628.

67. Longmore DB, Hoyle PM, Gregory A, et al. Prostacyclin administration during cardiopulmonary bypass in man. Lancet 1981;1:800-803.

68. van den Dungen JJ, Karliczek GF, Brenken U, et al. The effect of prostaglandin E1 in patients undergoing clinical cardiopulmonary bypass. Ann Thorac Surg 1983;35(4):406-414.

69. McKenna R, Bachmann F, Whittaker B, et al. The hemostatic mechanism after open-heart surgery. II. Frequency of abnormal platelet functions during and after extracorporeal circulation. J Thorac Cardiovasc Surg 1975;70:298-308.

70. Coppe D, Sobel M, Seamans L, et al. Preservation of platelet function and number by prostacyclin during cardiopulmonary bypass. J Thorac Cardiovasc Surg 1981;81:274-278.

71. Mohr R, Golan M, Martinowitz U, et al. Effect of cardiac operation on platelets. J Thorac Cardiovasc Surg 1986;92:434-441.

72. Jones DK, Luddington R, Higenbottam TW, et al. Changes in factor VIII proteins after cardiopulmonary bypass in man suggest endothelial damage. Thromb. Haemostas. 1988;60:199-204.

73. Hackmann T, Gascoyne RD, Naiman SC, et al. A trial of desmopressin (1-desamino-8-D-arginine vasopressin) to reduce blood loss in uncomplicated cardiac surgery. N Engl J Med 1989;321:1437-1443.

74. Harker LA,Slichter SJ. The bleeding time as a screening test for evaluation of platelet function. N Engl J Med 1972;287:155-159.

75. Ekert H, Dowling SV. Platelet release abnormality and reduced prothrombin levels in children with cyanotic congenital heart disease. Aust Paediatr J 1977;13:17-21.

76. Maurer HM, McCue CM, Caul, et al. Impairment in platelet aggregation in congenital heart disease. Blood 1972;40:207-211.

77. Kowalski E, Kopec M, Wegrzynowicz Z. Influence of fibrinogen degradation products (FDP) on platelet aggregation, adhesiveness, and viscous metamorphis. Thromb Diath Haemorrh1963;10:406-413.

78 Ray MJ, Marsh NA, Hawson GA. Relationship of fibrinolysis and platelet function to bleeding after cardiopulmonary bypass. Blood Coagul Fibrinolysis 1994;5(5):679-685.

79. Wallace HW, Liquior EM, Stein TP, et al. Denatured of plasma platelet function. Trans Am Soc Artif Intern Organs 1975;21:450-455.

80. Lee WH, Krumhaar D, Fonkalsrud E, et al. Denaturation of plasma proteins as the cause of morbidity and death after intra cardiac operation. Surgery 1961;50:29-39.

81. Pruitt KM, Stroud RM, Scott JW. Blood damage in the heart-lung machine. Proc Soc Exp Biol Med 1971;137:714-718.

82. George JN, Thoi LL, Morgan RK. Quantitative analysis of platelet membrane glycoproteins: Effect of platelet washing procedures and isolation of platelet density subpopulations. Thromb Res 1981;23:69-77.

83. Levine SP, Krentz LS. Development of a radioimmunoassay for human platelet factor 4. Thromb Res 1977;11:673-686.

84. Michelson AD. Review article. Flow cytometry: a clinical test of platelet function. Blood 1996;87:4925-4936.

85. Hansen SR, Harker LA. Interruption of acute platelet-dependent thrombosis by the synthetic antithrombin PPACK. Proc Natl Acad Sci 1988;85:3184-3188.

86. Eidt JF, Allison P, Nobel S, et al. Thrombin is an important mediator of platelet aggregation in stenosed canine coronary arteries with endothelial injury. J Clin Invest 1989;84:18-27.

87. Kelly AB, UM Marzec, W Krupski, et al. Hirudin interruption of heparin-resistant arterial thrombus formation in baboons. Blood 1991;77:1006-1012.

88. Khuri SF, Valeri CR, Loscalzo J, et al. Heparin causes platelet dysfunction and induces fibrinolysis before cardiopulmonary bypass. Ann Thorac Surg 1995;60(4):1008-1014.

89. Upchurch GR, Valeri CR, Khuri SF, et al. The effect of heparin on fibrinolytic activity and platelet function in vivo. Am J Physiol 996;271:H528-534.

90. Michelson AD, MacGregor H, Barnard MR, et al. Reversible inhibition of human platelet activation by hypothermia in vivo and in vitro. Thromb Haemost 1994;71:633-640.

91. Van Overen W, Harder MP, Roozendaal KJ, et al. Aprotinin protects platelets against the

initial effect of cardiopulmonary bypass. J Thorac Cardiovasc Surg 1990;99:788-796.

92. Wenger RK, Lukasiewicz H, Mikuta BS, et al. Loss of platelet fibrinogen receptors during clinical cardiopulmonary bypass. J Thorac Cardiovasc Surg 1989;97:235-239.

93. Dechavanne M, French M, Pages J, et al. Significant reduction in the binding of a monoclonal antibody (LYP 18) directed against the IIb-IIIa glycoprotein complex to platelets of patients having undergone extracorporeal circulation. Thromb Haemost 1987;57:106-109.

94. Vander Salm TJ, Kaur S, Lancey RA, et al. Reduction of bleeding after heart operations through the prophylactic use of epsilon-amino caproic acid. J Thorac Cardiovasc Surg 1996;112:1098-1107.

95. Rinder CS, Mathew JP, Rinder HM, et al. Modulation of platelet surface adhesion receptors during cardiopulmonary bypass. Anesthesiology 1991;75:563-570.

96. Rinder CS, Bonan JL, Rinder HM, et al. Cardiopulmonary bypass induces leukocyte-platelet adhesion. Blood 1992;79:1201-1205.

97. Komai H, Haworth SG. Effect of cardiopulmonary bypass on the circulating level of soluble GMP-140. Ann Thorac Surg 1994;58:478-482.

98. Michelson AD, Barnard MR, Hechtman HB, et al. In vivo tracking of platelets: circulating degranulated platelets rapidly lose surface P-selectin but continue to circulate and function. Proc Natl Acad Sci USA 1996;93:11877-11882.

99. Levine SP. Secreted platelet proteins as markers for pathological disorders. In: Biochemistry of platelets. Phillips DR and Shuman MA, eds. Academic Press, Orlando. 1986;378-415.

100. Abrams CS, Ellison N, Budzynski AZ, et al. Direct detection of activated platelets and platelet-derived micro particles in humans. Blood 1990;75:128-138.

101. Chenoweth DE, Cooper SW, Hugli TE, et al. Complement activation during cardiopulmonary

bypass: evidence for generation of C3a and C5a anaphylatoxins. N Engl J Med 1981;304:497-503.

102. Gotta AW, Carsons S, Abrams L, et al. Fibronectin levels during cardiopulmonary bypass. New York State J Med 1987;87:493-495.

103. Saba TM, Jaffe E. Plasma fibronectin (opsonic glycoprotein): its synthesis by vascular endothelial cells and role in cardiopulmonary integrity after trauma as related to reticuloendothelial function. Am J Med 1980;68:577-594.

104. Pourrat E, Sie PM, Desrez X, et al. Changes in plasma fibronectin levels after cardiac and pulmonary surgery: role of cardiopulmonary bypass. Scand J Thorac Cardiovasc Surg 1985;19:63-67.

105. Deno DC, McCafferty MH, Saba TM, et al. Mechanism of acute depletion of plasma fibronectin following thermal injury in rats. Appearance of a gelatin like ligand in plasma. J Clin Invest 1984; 73:20-34.

106. Meade TW, North WR, Chakrabarti R, et al. Haemostatic function and cardiovascular death: Early results of a prospective study. Lancet 1980;1:1050-1054.

107. Lowe GDO, Drummond MM, Lorimer AR, et al. Relation between extent of coronary artery disease and blood viscosity. Br Med J 1980;8:673-674.

108. DiMinno G, Mancini M. Measuring plasma fibrinogen to predict stroke and myocardial infarction. Arteriosclerosis 1990;10:1-7.

109. Woods JE, Kirklin JW, Owen CA, et al. Effect of bypass surgery on coagulation-sensitive clotting factors. Mayo Clin Proc 1967;42:724-735.

110. Douglas AS, McNicol GP, Bain WH, et al. The hemostatic defect following extracorporeal circulation. Br J Surg 1966;53:455-467.

111. Gralnick HR, Fischer RD. The hemostatic response to open-heart operations. J Thorac

Cardiovasc Surg 1971;61:909-915.

112. Mustard JF, Hoeksema TD, Mustard WT, et al. Activity of the clotting mechanism during the extracorporeal shunting of blood. Bibl Haemat 1962;13:84-89.

113. Holloway DS, Summaria L, Sandesara J, et al. Decreased platelet number and function and increased fibrinolysis contribute to postoperative bleeding in cardiopulmonary bypass patients. Thromb Haemostas 1988;59(1):62-67.

114. Stibbe J, Kluft C, Brommer EJ, et al. Enhanced fibrinolytic activity during cardiopulmonary bypass in open-heart surgery in man is caused by extrinsic (tissue-type) plasminogen activator. Eur J Clin Invest 1984;14(5):375-382.

115. Mori F, Nakahara Y, Kurata S, et al. Late changes in hemostatic parameters following open-heart surgery. J Cardiovasc Surg (Torino) 1982;23(6):458-462.

116. Blauhut B, Gross C, Necek S, et al. Effects of high-dose aprotinin on blood loss, platelet function, fibrinolysis, complement, and renal function after cardiopulmonary bypass. J Thorac Cardiovasc Surg 1991;101(6):958-967.

117. Tanaka K, Wada K, Morimoto T, et al. The role of the protein C-thrombomodulin system in physiologic anticoagulation during cardiopulmonary bypass. ASAIO Trans 1989;35(3):373-375.

118. Kucuk O, Kwaan HC, Frederickson J, et al. Increased fibrinolytic activity in patients undergoing cardiopulmonary bypass operation. Am J Hematol 1986;23(3):223-229.

119. Sukhanov VA, Bauman NN, Levit AL. Hyperfibrinolysis: its express diagnosis and role in the development of haemorrhagic syndrome after cardiopulmonary bypass. Cor Vasa 1988;30(6):442-446.

120. Gando S, Tedo I, Goda Y, et al. [Increased fibrinolytic activity during surgery with cardiopulmonary bypass] Masui 1990;39(6):751-756. (Published in Japanese)

121. Kongsgaard UE, Smith-Erichsen N, Geiran O, et al. Changes in the coagulation and fibrinolytic systems during and after cardiopulmonary bypass surgery. Thorac Cardiovasc Surg 1989; 37(3):158-162.

122. Kongsgaard UE, Smith-Erichsen N, Geiran O, et al. Changes in the plasma protein systems during open-heart surgery with dextran vs. Ringer acetate as priming solution. Scand J Thorac Cardiovasc Surg 1989;23(3):253-258.

123. Giuliani R, Szwarcer E, Martinez Aquino E, et al. Fibrin-dependent fibrinolytic activity during extracorporeal circulation. Thromb Res 1991;61(4):369-373.

124. Valen G, Erikson E, Risberg B, et al. Fibrinolysis during cardiac surgery. Release of tissue plasminogen activator in arterial and coronary sinus blood. Eur J Cardiovasc Surg 1994;8(6):324-330.

125. Lambert CJ, Marengo-Rowe AJ, Leveson JE, et al. The treatment of postperfusion bleeding using epsilon-amino caproic acid, cryoprecipitate, fresh-frozen plasma, and protamine sulfate. Ann Thorac Surg 1979;28(5):440-444.

126. Stead R. Comparison of two methods for heparin monitoring: a semi-automated heparin monitoring device and activated clotting time during extracorporeal circulation. Int J Clin Monit Comput 1989;6(4):247-254.

127. Bachmann F, Parisi P. Fibrinolysis. In: Friedel N, Hetzer R, Royston D, eds. Blood use in cardiac surgery. Darmstadt: Steinkopff Verlag, 1991:3-9.

128. Kluft C. Pathomechanisms of defective hemostasis during and after extracorporeal circulation: Contact phase activation. In: Friedel N, Hetzer R, Royston D, eds. Blood use in cardiac surgery. Darmstadt: Steinkopff Verlag, 1991:10-15.

129. Chung JH, Gikakis N, Rao AK, et al. Pericardial blood activates the extrinsic coagulation pathway during clinical cardiopulmonary bypass. Circulation 1996;93(11):2014-2018.

130. Tabuchi N, de Haan J, Boonstra PW, et al. Activation of fibrinolysis in the pericardial cavity

during cardiopulmonary bypass. J Thorac Cardiovasc Surg 1993;106(5);828-833.

131. Brody KI, Pickering NJ, Fink GB. Concentrations of factor VIII-related antigen and factor XIII during open heart surgery. Transfusion 1986;26:478-480.

132. Weinstein M, Ware JA, Troll J, et al. Changes in von Willebrand factor during cardiac surgery: effect of desmopressin acetate. Blood 1988;71:1648-1655.

133. Gill JG, Wilson AD, Endres-Brooks J, et al. Loss of the largest von Willebrand factor multimers from the plasma of patients with congenital cardiac defects. Blood 1986;67:758-761.

134. Butler J, Rocker GM, Westaby S. Inflammatory response to cardiopulmonary bypass. Ann Thorac Surg 1993;55(2):552-559.

135. Edmunds JH Jr. Why cardiopulmonary bypass makes patients sick: Strategies to control the blood-synthetic surface interface. In: Advances in Cardiac surgery. Mosby-Year Book, Inc. 1995;6:131-167.

136. Rinder CS, Rinder HM, Smith BR, et al. Blockade of C5a and C5b-9 generation inhibits leukocyte and platelet activation during extracorporeal circulation.. J Clin Invest 1995;96:1564-1572.

137. Hennein HA, Ebba H, Rodriguez JL, et al. Relationship of the proinflammatory cytokines to myocardial ischemia and dysfunction after uncomplicated coronary revascularization. J Thorac Cardiovasc Surg 1994;108(4):626-635.

138. Steinberg JB, Kapelanski DP, Olson JD, et al. Cytokine and complement levels in patients undergoing cardiopulmonary bypass. J Thorac Cardiovasc Surg 1993;106(6):1008-1016.

139. Kawamura T, Wakusawa R, Okada K, et al. Elevation of cytokines during open heart surgery with cardiopulmonary bypass: participation of interleukin 8 and 6 in reperfusion injury. Can J Anesth 1993;40(11):1016-1021.

140. Seghaye MC, Duchateau J, Grabitz RG, et al. Effect of sodium nitroprusside on complement activation induced by cardiopulmonary bypass: a clinical and experimental study. J Thorac Cardiovasc Surg 1996:111(4):882-292.

141. Ovrum E, Mollnes TE, Fosse E, et al. High and low heparin dose with heparin-coated cardiopulmonary bypass: activation of complement and granulocytes. Ann Thorac Surg 1995;60(6):1755-1761.

142. Pina-Cabral JM, Ribeiro-da-Silva A, Almeida-Dias A. Platelet sequestration during hypothermia in dogs treated with sulphinpyrazone and ticlopidine-reversibility accelerated after intra-abdominal rewarming. Thromb Haemost 1985;54(4):838-841.

143. Yoshihara H, Yamamoto T, Mihara H. Changes in coagulation and fibrinolysis occurring in dogs during hypothermia. Thromb Res 1985;37(4):503-512.

144. Moore FD Jr, Warner KG, Assousa S, et al. The effect of complement activation during cardiopulmonary bypass. Attenuation by hypothermia, heparin and hemodilution. Ann Surg 1988;208(1):95-103.

145. Hirsh J. Heparin. New Engl J Med 1991;324(22):1565-1574.

146. Olsson P, Lagergren H, Ek S. The elimination from plasma of intravenous heparin: an experimental study on dogs and humans. Acta Med Scand 1963;173:619-630.

147. Bjornsson TO, Wolfram BS, Kitchell BB. Heparin kinetics determined by three assay methods. Clin Pharmacol Ther 1982;31:104-113.

148. Habbab MA, Haft JI. Heparin resistance induced by intravenous nitroglycerin. Circulation 1986;74:suppl II:II-321, abstract.

149. Pizulli L, Nitsch J, Luderitz B. Inhibition of the heparin effect by nitroglycerin. Dtsch Med Wochenschr 1988;133:1837-1840.

150. Lefemine AA, Lewis M. Activated clotting time for control of anticoagulation during surgery. Am Surg 1985;51(5):274-278.

151. Tuman KJ, Spiess BD, McCarthy RJ, et al. Comparison of viscoelastic measures of coagulation after cardiopulmonary bypass. Anesth Analg 1989;69(1):69-75.

152. Gravlee GP, Whitaker CL, Mark LJ, et al. Baseline activated coagulation time should be measured after surgical incision. Anesth Analog 1990;71(5):549-553.

153. Ovrum E, Lindberg H, Holen EA, et al. Systemic and pulmonary circulatory effects of protamine following cardiopulmonary bypass in man. Scand J Thorac Cardiovasc Surg 1991;25(1):19-24.

154. Procaccini B, Clementi G, Bersanetti L, et al. Cardiorespiratory Effects of protamine sulphate in man: intra-aortic vs intra-right atrial rapid administration after cardiopulmonary bypass. J Thorac Surg (Torino) 1987;28(2):112-119.

155. Katz NM, Kim YD, Siegelman R, et al. Hemodynamics of protamine administration. Comparison of right atrial, left atrial, and aortic injections. J Thorac Cardiovasc Surg 1987;94(6):881-886.

156. Whitman GJ, Martel D, Weiss M, et al. Reversal of protamine-induced catastrophic pulmonary vasoconstriction by prostaglandin E1. Ann Thorac Surg 1990;50(2):303-305.

157. Kirklin JK, Chenoweth DE, Naftel DC, et al. Effects of protamine administration after cardiopulmonary bypass on complement, blood elements, and the hemodynamic state. Ann Thorac Surg 1986;41(2):193-199.

158. Westaby S, Turner MW, Stark J. Complement activation and anaphylactoid response to protamine in a child after cardiopulmonary bypass. Br Heart J 1985;53(5):574-576.

159. Weiler JM, Gellhaus MA, Carter JG, et al. A prospective study of the risk of an immediate

adverse reaction to protamine sulfate during cardiopulmonary bypass surgery. J Allergy Clin Immunol 1990;85(4):713-719.

160. Cavarocchi NC, Schaff HV, Orszulak TA, et al. Evidence for complement activation by protamine-heparin interaction after cardiopulmonary bypass. Surgery 1985;98(3):525-531.

161. Sharath MD, Metzer WJ, Richerson HB, et al. Protamine-induced fatal anaphylaxis. Prevalence of antiprotamine immunoglobulin E antibody. J Thorac Cardiovasc Surg 1985;90(1):86-90.

162. Wolk LA, Wilson RF, Burdick M, et al. Changes in antithrombin, antiplasmin, and plasminogen during and after cardiopulmonary bypass. Am Surg 1985;51(6):309-313.

163. Uziel L, Cugno M, Cacciabue E, et al. Evaluation of tests for heparin control during long-term extracorporeal circulation. Int J Artif Organs 1986;9(2):111-116.

164 Uziel L, Cugno M, Fabrizi I, et al. Physiopathology and management of coagulation during long-term extracorporeal respiratory assistance. Int J Artif Organs 1990;13(5):280-287.

165. Despotis GJ, Summerfield AL, Joist JH, et al. Comparison of activated coagulation time and whole blood heparin measurements with laboratory plasma anti-Xa heparin concentration in patients having cardiac operations. J Thorac Cardiovasc Surg 1994;108(6):1076-1082.

166. Wang JS, Lin CY, Karp RB. Comparison of high-dose thrombin time with activated clotting time for monitoring of anticoagulant effects of heparin in cardiac surgical patients. Anesth Analg 1994;79(1):9-13.

167. Rohrer MJ, Natale AM. The effect of hypothermia on the coagulation cascade. Crit Care Med 1992;20(10):1402-1405.

168. Despotis GJ, Santoro SA, Spitznagel E, et al. Prospective evaluation and clinical utility of on-site monitoring of coagulation in patients undergoing cardiac operation. J Thorac Cardiovasc Surg 1994;107(1):271-279.

169. Despotis GJ, Joist JH, Hogue CW Jr, et al. The impact of heparin concentration and activated clotting time monitoring on blood conservation. A prospective, randomized evaluation in patients undergoing cardiac operations. J Thorac Cardiovasc Surg 1995;110(1):46-54.

170. Hardy JF, Belisle S, Robitaille D, et al. Measurement of heparin concentration in whole blood with the Hepcon. HMS device does not agree with laboratory determination of plasma heparin concentration using a chromogenic substrate for activated factor X. J Thorac Cardiovasc Surg 1996;112(1):154-161.

171. Jobes DR, Aitken GL, Shaffer GW. Increased accuracy and precision of heparin and protamine dosing reduces blood loss and transfusion in patients undergoing primary cardiac operations. J Thorac Cardiovasc Surg 1995;110(1):36-45.

172. Gorman RC, Ziats N, Rao AK, et al. Surface-bound heparin fails to reduce thrombin formation during clinical cardiopulmonary bypass. J Thorac Cardiovasc Surg 1996;111(1):1-11.

173. Brister SJ, Ofosu FA, Buchanan MR. Thrombin generation during cardiac surgery: is heparin the ideal anticoagulant? Thromb Haemost 1993;70(2):259-262.

174. Shanberge JN, Murato M, Quattrociocchi-Longe T, et al. Heparin-protamine complexes in the production of heparin rebound and other complications of extracorporeal bypass procedures. Am J Clin Pathol 1987;87(2):210-217.

175. Teoh KH, Young E, Bradley CA, et al. Heparin binding proteins. Contributions to heparin rebound after cardiopulmonary bypass. Circulation 1993;88(5 Pt2):II420-425.

176. Gundry SR, Drongowski RA, Klein MD, et al. Postoperative bleeding in cardiovascular surgery. Does heparin rebound really exist? Am Surg 1989;55(3):162-165.

177. Berndt MC, Chong BH, Andrews RK. Biochemistry of drug-dependent platelet autoantigens. In: Kunicki TJ, George JN, eds. Platelet immunobiology. Molecular and clinical aspects. Philadelphia: JB Lippincott, 1989:132-147.

178. Salzman EW, Rosenberg RD, Smith MH, et al. Effect of heparin and heparin fractions on platelet aggregation. J Clin Invest 1980;65:64-70.

179. Makhoul RG, McCann RL, Austin EH, et al. Management of patients with heparin-associated thrombocytopenia and thrombosis requiring cardiac surgery. Ann Thorac Surg 1987;43(6):617-621.

180. Messmore HL. Clinical efficacy of heparin fractions: issues and answers. Crit Rev Clin Lab Sci 1986;23(2):77-94.

181. Dreyfus G, Massonnet-Castel S, Pelissier E, et al.(Low molecular weight heparin in extracorporeal circulation. 1st clinical applications.) Etude d'une heparine de bas poids moleculaire au cours des circulations extracorporelles. Premieres applications cliniques. Arch Mal Coeur 1986;79(13):1851-1857. (published in French)

182. Rowlings PA, Mansberg R, Rozenberg MC, et al. The use of a low molecular weight heparinoid (Org 10171) for extracorporeal procedures in patients with heparin dependent thrombocytopenia and thrombosis. Aust N Z J Med 1991;21(1):52-54.

183. Altes A, Martino R, Gari M, et al. Heparin-induced thrombocytopenia and heart operation: management with tedelparin. Ann Thorac Surg 1995;59(2):508-509.

184 Wilhelm MJ, Schmid C, Kececioglu D, et al. Cardiopulmonary bypass in patients with heparin-induced thrombocytopenia using Org 10172. Ann Thorac Surg 1996;61(3):920-924.

185. Teasdale SJ, Zulys VJ, Mycyk T, et al. Ancrod anticoagulation for cardiopulmonary bypass in heparin-induced thrombocytopenia and thrombosis. Ann Thorac Surg 1989;48(5):712-713.

186. Spiekermann BF, Lake CL, Rich GF, et al. Normal activated clotting time despite adequate anticoagulation with ancrod in a patient with heparin-associated thrombocytopenia and thrombis undergoing cardiopulmonary bypass. Anesthesiology 1994;80(3):686-688.

187. Walenga JM, Bakhos M, Messmore HL,et al. Potential use of recombinant hirudin as an

anticoagulant in a cardiopulmonary bypass model. Ann Thorac Surg 1991;51(2):271-277.

188. Koza MJ, Walenga JM, Fareed J, et al. A new approach in monitoring recombinant hirudin during cardiopulmonary bypass. Semin Thromb Hemaost 1993;19 Suppl !:90-96.

189. Schiele F, Vuillemenot A, Mouhat T, et al. Anticoagulant therapy with recombinant hirudin in patients with thrombopenia induced by heparin. Presse Med 1996;25(16):757-760.

190. Mollnes TE, Videm V, Gotze O, et al. Formation of C5a during cardiopulmonary bypass: inhibition by precoating with heparin. Ann Thorac Surg 1991;52(1):92-97.

191. von Segesser LK, Turina M. Cardiopulmonary bypass without systemic heparinization. Performance of heparin-coated oxygenators in comparison with classic membrane and bubble oxygenators. J Thorac Cardiovasc Surg 1989;98(3):386-396.

192. Tong SD, Rolfs MR, Hsu LC. Evaluation of Duraflo II heparin immobilized cardiopulmonary bypass circuits. ASAIO Trans 1990;36(3):M654-656.

193. Videm V, Nilsson L, Venge P, et al. Reduced granulocyte activation with a heparin-coated device in an in vitro model of cardiopulmonary bypass. Artif Organs 1991;15(2):90-95.

194. Nilsson L, Storm KE, Thelin S, et al. Heparin-coated equipment reduces complement activation during cardiopulmonary bypass in the pigs. Artif Organs 1990;14(1):46-48.

195. von Segesser LK, Weiss BM, Garcia E, et al. Reduced blood loss and transfusion requirements with low systemic heparinization: preliminary clinical results in coronary artery revascularization. Eur J Cardiothorac Surg 1990;4(2):639-643.

196. von Segesser LK, Turina M. Long term cardiopulmonary bypass without systemic heparinization. Int J Artif Organs 1990;13(10):687-691.

197. von Segessor LK, Weiss BM, Pasic M, et al. Experimental evaluation of heparin-coated

cardiopulmonary bypass equipment with low systemic heparinization and high-dose aprotinin. Thorac Cardiovasc Surg 1991;39(5):2510256.

198. Redmond JM, Gillinov AM, Stuart RS, et al. Heparin-coated bypass circuits reduce pulmonary injury. Ann Thorac Surg 1993;56(3):474-478.

199. Wendel HP, Heller W, Gallimore MJ, et al. Heparin-coated oxygenators significantly reduce contact system activation in an in vitro cardiopulmonary bypass model. Blood Coagul Fibrinolysis 1994;5(5):673-678.

200. Van der Kamp KW, van Oeveren W. Contact, coagulation and platelet interaction with heparin treated equipment during heart surgery. Int J Artif Organs 1993;16(12):836-842.

201. te Velthius H, Jansen PG, Hck Ce, et al. Specific complement inhibition with heparin-coated extracorporeal circuits. Ann Thorac Surg 1996;61(4):1153-1157.

202. Boonstra PW, Gu YJ, Akkerman C, et al. Heparin coating of an extracorporeal circuit partly improves hemostasis after cardiopulmonary bypass. J Thorac Cardiovasc Surg 1994;107(1):289-292.

203 Shigemitsu O, Hadama T, Takasaki H, et al. Biocompatibility of a heparin-bonded membrane oxygenator (Carmeda MAXIMA) during the first 90 minutes of cardiopulmonary bypass: clinical comparison with the conventional system. Artif Organs 1994;18(12):936-941.

204. Wagner WR, Johnson PC, Thompson KA, et al. Heparin-coated cardiopulmonary bypass circuits: hemostatic alterations and postoperative blood loss. Ann Thorac Surg 1994;58(3):734-740.

205. Muchrcke DD, McCarthy PM, Kottke-Merchant K, et al. Biocompatibility of heparin-coated extracorporeal bypass circuits: A randomized, masked clinical trial. J Thorac Cardiovasc Surg 1996;112(2):472-483.

206. Sellevold OF, Berg TM, Rein KA,et al. Heparin-coated circuit during cardiopulmonary bypass. A clinical study using closed-circuit, centrifugal pump and reduced heparinization. Acta Anaesthesiol

Scand 1994;38(4):372-379.

207 Jones DR, Hill RC, Vasilakis A, et al. Safe use of heparin-coated bypass circuits incorporating a pump-oxygenator. Ann Thorac Surg 1994;57(4):815-818.

208. Ovrum E, Brosstad F, Am Holen E, et al. Effects of coagulation and fibrinolysis with reduced versus full systemic heparinization and heparin-coated cardiopulmonary bypass. Circulation 1995;92(9):2579-2584.

209. Ovrum E, Holen EA, Tangen G, et al. Completely heparinized cardiopulmonary bypass and reduced systemic heparin: clinical and hemostatic effects. Ann Thorac Surg 1995;60(2):365-371.

210. Aldea GS, Doursounian M, O'Gara P, et al. Heparin-bonded circuits with a reduced anticoagulation protocol in primary CABG: a prospective, randomized study. Ann Thorac Surg 1996;62(2):410-417.

211. Von Segesser LK, Weiss BM, Pasic M, et al. Risk and benefit of low systemic heparinization during open heart operations. Ann Thorac Surg 1994;58(2):391-397.

212. Messmore HL. Clinical potential of low molecular weight heparins. Sem Thromb Hemost 1989;15(4):405-408.

213. Blajchman MA, Young E, Osofu FA. Effects of unfractionated heparin, dermatan sulfate and low molecular weight heparin on vessel wall permeability in rabbits. Ann N Y Acad Sci 1989;556:245-254.

214. Hirsh J. From unfractionated heparins to low molecular weight heparins. Acta Chir Scand Suppl 1990;556:42-50.

215. Henny CP, Ten Cate H, Ten Ctae JW, et al. A randomized blind study comparing standard heparin and a new low molecular weight heparinoid in cardiopulmonary bypass surgery in dogs. J Lab Clin Med 1985;106(2):187-196.

216. Reber G, Schweizer A, de Moerloose O, et al. Comparison between a low molecular weight and standard heparin for anticoagulation during extracorporeal CO_2 removal in the dog. Thromb Res 1988;49(2):157-168.

217. Doherty DC, Ortel TL, de Bruijn N, et al. "Heparin-free" cardiopulmonary bypass: first reported use of heparinoid (Org 10172) to provide anticoagulation for cardiopulmonary bypass. Anesthesiology 1990;73(3):562-565.

218. Bagge L, Holmer E, Wahlberg T, et al. Fragmin (LMWH) vs heparin for anticoagulation during in vitro recycling of human blood in cardiopulmonary bypass circuits: dose-dependence and mechanisms of clotting. Blood Coagul Fibrinolysis 1994;5(2):273-280.

219. Koza MJ, Messmore HL, Wallock ME, et al. Evaluation of a low molecular weight heparin as an anticoagulant in a model of cardiopulmonary bypass surgery. Thromb Res 1993;70(1):67-76.

220. Terrell MR, Walenga JM, Koza MJ, et al. Efficacy of aprotinin with various anticoagulant agents in cardiopulmonary bypass. Ann Thorac Surg 1996;62(2):506-511.

221 Bagge L, Wahlberg T, Holmer E, et al. Low-molecular-weight heparin (Fragmin) versus heparin for anticoagulation during cardiopulmonary bypass in open heart surgery, using a pig model. Blood Coagul fibrinolysis 1994;5(2):265-272.

222. Weitz JI, Hudoba M, Massel D, et al. Clot-bound thrombin is protected from inhibition by heparin-antithrombin III but is susceptible to inactivation by antithrombin III-independent inhibitors. J Clin Invest 1990;86:385-391.

223. Heras M, Chesebro JH, Penny WJ, et al. Effects of thrombin inhibition on the development of acute platelet-thrombus deposition during angioplasty in pigs: heparin versus recombinant hirudin, a specific thrombin inhibitor. Circulation 1989;79:657-665.

224. Agnelli G, Pascucci C, Cosmi B, et al. The comparative effects of recombinant hirudin (CGP 39393) and standard heparin on thrombus growth in rabbits. Thromb Haemost 1990;63:204-207.

225. Mathiasson SE, Lindblad B, Stjernquist U, et al. The haemorrhagic effect of low-dose molecular weight heparins, dermatan sulphate and hirudin. Haemostasis 1995;25(5):203-211.

226. Zoldhelyi P, Bicjhler J, Owen WG, et al. Persistent thrombin generation in humans during specific thrombin inhibition with hirudin. Circulation 1994;90(6):2671-2678.

227. Zulys VJ, Teasdale SJ, Michel ER, et al. Ancrod (Arvin) as an alternative to heparin anticoagulation for cardiopulmonary bypass. Anesthesiology 1989;71(6):870-877.

228. Smith RE, Townsend GE, Berry BR, et al. Enoxaparin for unstable angina and ancrod for cardiac surgery following heparin allergy. Ann Pharmacother 1996;30(5):476-480.

229. Brister SJ, Ofosu FA, Heigenhauser GJ, et al. Is heparin the ideal anticoagulant for cardiopulmonary bypass? Dermatan sulphate mat be an alternate choice. Thromb Haemost 1994;71:468-473.

230. DeAnda A Jr, Coutre SE, Moon MR, et al. Pilot studies of the efficacy of a thrombin inhibitor for use during cardiopulmonary bypass. Ann Thorac Surg 1994;58(2):344-350.

231. Chomiak PN, Walenga JM, Koza MJ, et al. Investigation of a thrombin inhibitor peptide as an alternative to heparin in cardiopulmonary bypass surgery. Circulation 1993;88(5 Pt 2):II407-412.

232. Walenga JM, Koza MJ, Park SJ, et al. Evaluation of CCP 39393 as the anticoagulant in cardiopulmonary bypass operation in a dog model. Ann Thorac Surg 1994;58(6): 1685-1689.

233. Linhardt RJ, Langer R. New approaches for anticoagulation in extracorporeal therapy. Biomater Artif Cells Artif Organs 1987;15(1):91-100.

234. Yang VC, Teng CL, Kim JS. A filter device for the prevention of both heparin- and protamine-induced complications associated with extracorporeal therapy. Biomed Instrum Technol 1990;24(6):433-439.

235. Yang VC, Port FK, Kim JS, et al. The use of immobilized protamine in removing heparin and preventing protamine-induced complications during extracorporeal blood circulation. Anesthesiology 1991;75(2):288-297.

236. Hiramatsu Y, Gikakis N, Anderson HL, et al. Tirofiban provides "platelet anesthesia" during cardiopulmonary bypass in baboons. J Thorac Cardiovasc Surg 1997;113:182-193.

237. Williams RD, D'Ambra MN, Maione TE, et al. Recombinant platelet factor 4 reversal of heparin in human cardiopulmonary bypass blood. J Thorac Cardiovasc Surg 1994;108(5):975-983.

238. Bernabi A, Gilkakis N, Maione TE, et al. Reversal of heparin anticoagulation by recombinant platelet factor 4 and protamine sulfate in baboons during cardiopulmonary bypass. J Thorac Cardiovasc Surg 1995;109(4):765-771.

239. Levy JH, Cormack JG, Morales A. Heparin neutralization by recombinant platelet factor 4 and protamine. Anesth Analg 1995;81(1):35-37.

240. Evans DA, Holder RL, Brawn WJ, et al. Post-operative blood loss following cardiopulmonary bypass in children. Eur J Cardiothorac Surg 1994;8(1):25-29.

241. Despotis GJ, Filos KS, Zoys TN, et al. Factors associated with excessive postoperative blood loss and hemostatic transfusion requirements: a multivariate analysis in cardiac surgical patients. Anesth Analg 1996;82(1):13-21.

242 Tonz M, Mihaljevic T, von Segesser LK, et al. Normothermia versus hypothermia during cardiopulmonary bypass: a randomized, controlled trial. Ann Thorac Surg 1995;59(1):137-143.

243. Gelb AB, Roth RI, Levin J, et al. Changes in blood coagulation during and following cardiopulmonary bypass: lack of correlation with clinical bleeding. Am J Clin Pathol 1996;106(1):87-99.

244. Gravlee GP, Arora S, Lavender SW, et al. Predictive value of blood clotting tests in cardiac

surgery patients. Ann Thorac Surg 1994;58(1):216-221.

245. Burns ER, Billet HH, Frater RWM, et al. The preoperative bleeding time as a predictor of postoperative hemorrhage after cardiopulmonary bypass. J Thorac Cardiovasc Surg 1986;92(2):310-312.

246. Marengo-Rowe AJ, Lambert CJ, Levenson JE, et al. The evaluation of hemorrhage in cardiac patients who have undergone extracorporeal circulation. Transfusion 1979;19:426-433.

247. Lind SE. The bleeding time does not predict surgical bleeding. Blood 1991;77(12):2547-2552.

248. Pillgram-Larsen J, Wisloff F, Jorgensen JJ, et al. Effect of high dose ampicillin and cloxacillin on bleeding time and bleeding in open-heart surgery. Scand J Thorac Cardiovasc Surg 1985;19:45.

249. Ramsey G, Arvan DA, Stewart S, et al. Do preoperative laboratory tests predict blood transfusion needs in cardiac operations? J Thorac Cardiovasc Surg 1983;85:564.

250. Ratnatunga CP, Rees GM, Kovacs IB. Preoperative hemostatic activity and excessive bleeding after cardiopulmonary bypass. Ann Thorac Surg 1991;52:250-257.

251. Ferraris VA, Berry W, Lough F. Routine template bleeding time determinations before cardiac procedures. J Thorac Cardiovasc Surg 1987;93:474-475.

252. Ferraris VA, Gildengorin V. Predictors of excessive blood use after coronary artery bypass grafting. J Thorac Cardiovasc Surg 1989;98:492-497.

253. Weksler BB, Pett SB, Alonso D, et al. Differential inhibition by aspirin of vascular and platelet prostaglandin synthesis in atherosclerotic patients. N Engl J Med 1983;308(14):800-805.

254 Goldman S, Copeland J, Moritz T, et al. Improvement in early saphenous vein graft patency after coronary artery bypass surgery with antiplatelet therapy. Circulation 1988;77:1324-1332.

255. Valeri CR, MacGregor H, Pompei F, et al. Letter to the Editor: "Acquired abnormalities of

platelet function". N Engl J Med 1991;324(23):1670.

256. Woodman RC, Harker LA. Bleeding complications associated with cardiopulmonary bypass. Blood 1990;76:1680-1697.

257. Brandt JT. Editorial: The role of hemostasis laboratory in cardiopulmonary bypass surgery. Am J Clin Pathol 1996;106(1):3-5.

258. Nuttall GA, Oliver WC, Beynen FM, et al. Determination of normal versus abnormal activated partial thromboplastin time and prothrombin time after cardiopulmonary bypass. J Cardiothorac Vasc Anesth 1995;9(4):355-361.

259. Nuttall GA, Oliver WC Jr, Beynen FM, et al. Intraoperative measurement of activated partial thromboplastin time and prothrombin time by a portable laser photometer in patients following cardiopulmonary bypass. J Cardiothorac Vasc Anesth 1993;7(4):402-409.

260. Rappaport SI. Preoperative hemostasis evaluation: Which test, if any? Blood 1983,61(2):229-231.

261. Magovern JA, Sakert T, Benckart DH, et al. A model for predicting transfusion after coronary artery bypass grafting. Ann Thorac Surg 1996;61:27-32.

262. Schmoeckel M, Nollert G, Mempel M, et al. Effects of recombinant human erythropoietin on autologous blood donation before open heart surgery. Thor Cardiovasc Surg 1992;41:364-368.

263. Watanabe Y, Fuse K, Naruse Y, et al. Subcutaneous use of erythropoietin in heart surgery. Ann Thorac Surg 1992;54:479-483.

264. Kulier AH, Gombotz H, Fuchs G, et al. Subcutaneous recombinant human erythropoietin and autologous blood donation before coronary artery bypass surgery. Anesth Analg 1993;76:102-106.

265. Konishi T, Ohbayashi T, Kaneko T, et al. Preoperative use of erythropoietin for cardiovascular

operations in anemia. Ann Thorac Surg 1993;56:101-103.

266 Hayashi J, Kumon K, Takanashi S. et al. Subcutaneous administration of recombinant human erythropoietin before cardiac surgery: A double blind, multicenter trial in Japan. Transfusion 1994;34:142-146.

267. Rosengart TK, DeBois B, Helm R, et al. Retrograde autologous priming (RAP) for cardiopulmonary bypass: a safe and effective means of decreasing hemodilution and transfusion requirements. Circulation 1995;92(8):suppl I:I-763.

268. Schonberger JP, Everts PA, Hoffman JJ. Systemic blood activation with open and closed venous reservoirs. Ann Thorac Surg 1995;59(6):1549-1555.

269. Love TR, Hendren WG, O'Keefe DD, et al. Transfusion of predonated autologous blood in elective cardiac surgery. Ann Thorac Surg 1987;43:508-512.

270. Gluck D, Kubanek B, Ahnefeld FW. Autologous transfusion. Objectives and benefits, limits and risks shown in a practical clinical concept. Anaesthesia 1988;37:565-571.

271. Anderson BV, Tomasulo PA. Current autologous transfusion practices. Implications for the future. Transfusion 1988;28:394-396.

272. Cohn LH, Fosbert AM, Anderson WP, et al. The effect of phlebotomy, hemodilution and autologous transfusion on systemic oxygenation and whole blood utilization in open heart surgery. Chest 1975;68:283-287.

273. Kaplan JA, Cannarella C, Jones EL, et al. Autologous blood transfusion during cardiac surgery. J Thorac Cardiovasc Surg 1977;74:4-10.

274. Moran JM, Babka R, Silverman S, et al. Immediate centrifugation of oxygenator contents after cardiopulmonary bypass. J Thorac Cardiovasc Surg 1978;76:510-517.

275. Pliam MB, McGoon DC, Tarhan S. Failure of transfusions of autologous whole blood to reduce banked-blood requirements in open-heart surgical patients. J Thorac Cardiovasc Surg 1975;70:338-343.

276. Sherman MM, Dobnik DB, Dennis RC, et al. Prediction of hematocrit changes in open heart surgery without blood transfusion. J Cardiovasc Surg (Torino) 1984;25:545-548.

277. Platelet Transfusion Therapy; National Institute of Health Consensus Development Conference Statement. Volume 6, Number 7, October 8, 1986.

278. Giordano GF, Rivers SL, Chung GKT, et al. Autologous platelet-rich plasma in cardiac surgery: Effect on intraoperative and postoperative transfusion requirements. Ann Thorac Surg 1988;46:416-419.

279. DelRossi AJ, Cernaianu AC, Vertrees RA, et al. Platelet-rich plasma reduces postoperative blood loss after cardiopulmonary bypass. J Thorac Cardiovasc Surg 1990;100:281-286.

280. Jones JW, McCoy TA, Rawitscher RE, et al. Effects of intraoperative plasmapheresis on blood loss in cardiac surgery. Ann Thorac Surg 1990;49:585-590.

281. Boldt J, von Bormann B, Kling D, et al. Preoperative plasmapheresis in patients undergoing cardiac surgery procedures. Anesthesiology 1990;72:282-288.

282. Schaff HV, Hauer JM, Bell WR, et al. Autotransfusion of shed mediastinal blood after cardiac surgery. J Thorac Cardiovasc Surg 1978;75:632-641.

283. Johnson RG, Rosenkrantz KR, Preston RA, et al. The efficacy of postoperative autotransfusion in patients undergoing cardiac operations. Ann Thorac Surg 1983;36:173-179.

284. Griffith LD, Billman GF, Daily PO, et al. Apparent coagulopathy caused by infusion of shed mediastinal blood and its prevention by washing of the infusate. Ann Thorac Surg 1989;47:400-406.

285. Thurer RI, Lytle BW, Cosgrove DM, et al. Autotransfusion following cardiac operations: A randomized prospective study. Ann Thorac Surg 1979;27:500-507.

286. Axford TC, Dearani JA, Ragno G, et al. The safety and effectiveness of reinfused shed blood after open heart surgery. Ann Thorac Surg 1993;57:615-622.

287. Valeri CR. Physiology of blood transfusion. In: Shires GT, Barie PS, eds. Surgical Intensive care. Little, Brown, Boston. 1993:681-721.

288 Healey N, Khuri SF, Valeri CR, et al. Platelets stored at -80°C for 1.3 years: A new option for post cardiopulmonary bypass transfusion therapy. Circulation 1995;92(8)suppl:I-764.

289. Fish KJ, Sarnquist FH, van Steennis C, et al. A prospective, randomized study of the effects of prostacyclin on platelets and blood loss during coronary bypass operations. J Thorac Cardiovasc Surg 1986;91:436-442.

290 Malpass TW, Amory DW, Harker LA, et al. The effect of prostacyclin infusion on platelet hemostatic function in patients undergoing cardiopulmonary bypass. J Thorac Cardiovasc Surg 1984,87(4):550-555.

291. Radegran K, Egberg N, Papaconstantinou C. Effects of prostacycylin during cardiopulmonary bypass in man. Scand J Thorac Surg 1981;15(3):263-268.

292. DiSesa VJ, Huval W, Lelcuk S, et al. Disadvantages of prostacyclin infusion during cardiopulmonary bypass: A double-blind study of 50 patients having coronary revascularization. Ann Thorac Surg 1984,38(5):514-519.

293. DelRossi AJ, Cernaiano AC, Botros S, et al. Prophylactic treatment of post-perfusion bleeding using epsilon-amino caproic acid. Chest 1989;96:27-30.

294. Penta de Peppo A, Pierri MD, Scafuri A, et al. Intraoperative antifibrinolysis and blood-saving techniques in cardiac surgery. Prospective trial of 3 antifibrinolytic drugs. Tex Heart Inst J 1995;22(3):231-236.

295. Arom KV, Emery RW. Decreased postoperative drainage with addition of epsilon-amino

caproic acid before cardiopulmonary bypass. Ann Thorac Surg 1994;57(5):1108-1112.

296. Okamoto S, Sato S, Takeda Y, et al. An active isomer (trans form) of AMCHA and its antifibrinolytic (antiplasminic) action in vitro and in vivo. Keio J Med 1964;13:177-185.

297. Horrow JC, Hlavacek J, Strong MD, et al. Prophylactic tranexamic acid decreases bleeding after cardiac operations. J Thorac Cardiovasc Surg 1990;99:70-74.

298. Shore-Lesserson L, Reich DL, Vela-Cantos F, et al. Tranexamic acid reduces transfusions and mediastinal drainage in repeat cardiac surgery. Anesth Analg 1996;83(1):18-26.

299. Karski JM, Teasdale SJ, Norman P, et al. Prevention of bleeding after cardiopulmonary bypass with high-dose tranexamic acid. Double-blind, randomized clinical trial. J Thorac Cardiovasc Surg 1995;110(3):835-842.

300. Nakashima A, Marsuzaki K, Fukumura F, et al. Tranexamic acid reduces blood loss after cardiopulmonary bypass. ASAIO J 1993;39(3):M185-189.

301. Rousou JA, Engelman RM, Flack JE 3rd, et al. Tranexamic acid significantly reduces blood loss associated with coronary revascularization. Ann Thorac Surg 1995;59(3):671-675.

302. Zonis Z, Seear M, Reichert C, et al. The effect of preoperative tranexamic acid on blood loss after cardiac operations in children. J Thorac Cardiovasc Surg 1996;111(5):9822-987.

303. Royston D, Bidstrup BP, Taylor KM, et al. Effect of aprotinin on need for blood transfusion after repeat open-heart surgery. Lancet 1987;ii:1289-1291.

304. Bidstrup BP, Royston D, Sapsford RN, et al. Reduction in blood loss and blood use after cardiopulmonary bypass with high dose aprotinin (Trasyslol). J Thorac Cardiovasc Surg 1989;97:364-372.

305. Lu H, Sorra C, Commin TL, et al. Haemostasis in patients undergoing extracorporeal circulation:

the effect of aprotinin (Trasylol). Thromb Haem 1991,66:633-637.

306. Dietrich W, Dilthey G, Spannagal M, et al. Influence of high-dose aprotinin on anticoagulation, heparin requirement, and celite- and kaolin-activated clotting time in heparin-pretreated patients undergoing open-heart surgery. A double-blind, placebo-controlled study. Anesthesiology 1995;83(4):679-689.

307. Dietrich W, Mossinger H, Spannagl M, et al. Hemostatic activation during cardiopulmonary bypass with different aprotinin dosages in pediatric patients having cardiac operations. J Thorac Cardiovasc Surg 1993;105(4):712-720.

308. Speekenbrink RG, Wildevuur CR, Sturk A, et al. Low-dose and high-dose aprotinin improve hemostasis in coronary operations. J Thorac Cardiovasc Surg 1996;112(2):523-530.

309 Lavee J, Raviv Z, Smolinsky A, et al. Platelet protection by low-dose aprotinin in cardiopulmonary bypass: electron microscopic study. Ann Thorac Surg 1993;55(1):114-119.

310. Bailey CR, Wielogorski AK. Randomized placebo controlled double blind study of two dose aprotinin regimens in cardiac surgery. Br Heart J 1994;71(4):349-353.

311. Liu B, Tengborn L, Larson G, et al. Half-dose aprotinin preserves hemostatic function in patients undergoing bypass operations. Ann Thorac Surg 1995;59(6):1534-1540.

312. Mastroroberto P, Chello M, Zofrea S, et al. Suppressed fibrinolysis after administration of low-dose aprotinin: reduced level of plasmin-alpha2-plasmin inhibitor complexes and postoperative blood loss. Eur J Cardiothorac Surg 1995;9(3):143-145.

313 Kawasuji M, Ueyama K, Sakakibara N, et al. Effect of low-dose aprotinin on coagulation and fibrinolysis in cardiopulmonary bypass. Ann Thorac Surg 1993;55(5):1205-1209.

314. Orchard MA, Goodchild CS, Prentice CRM, et al. Aprotinin reduces cardiopulmonary bypass-induced blood loss and inhibits fibrinolysis without influencing platelets. Brit J Haematol 1993;85:533-

541.

315. Lu H, DuBuit C, Soria J, et al. Postoperative hemostasis and fibinolysis in patients undergoing cardiopulmonary bypass with or without aprotinin therapy. Thromb Haemost 1994;72(3):438-443.

316. Tabuchi N, Huet RC, Sturk A, et al. Aprotinin preserves hemostasis in aspirin-treated patients undergoing cardiopulmonary bypass. Ann Thorac Surg 1994;58(4):1036-1039.

317. Wahba A, Black G, Koksch M, et al. Aprotinin has no effect on platelet activation and adhesion during cardiopulmonary bypass. Thromb Haemost 1996;75(5):844-848.

318. Ray MJ, Marsh NA, Just SJE, et al. Preoperative platelet dysfunction increases the benefit of aprotinin in cardiopulmonary bypass. Ann Thorac Surg 1997;63:57-63.

319. Wang JS, Lin CY, Hung WT, et al. In vitro effects of aprotinin on activated clotting time measured with different activators. J Thorac Cardiovasc Surg 1992;104(4):1135-1140.

320. Farooqi N, De Hert S, Vlaeminck R, et al. Effects of low doses of aprotinin on clotting times activated with celite and kaolin. Acta Anaesthesiol Belg 1993;44(3):87-92.

321. Despotis GJ, Joist JH, Joiner-Maier D, et al. Effect of aprotinin on activated clotting time, whole blood and plasma heparin measurements. Ann Thorac Surg 1995;59(1):106-111.

322. Huyzen RJ, Harder MP, Huet RC, et al. Alternative perioperative anticoagulation monitoring during cardiopulmonary bypass in aprotinin-treated patients. J Cardiothorac Vasc Anesth 1994;8(2):153-156.

323. de Smet AAEA, Joen MCN, van Oeveren W, et al. Increased anticoagulation during cardiopulmonary bypass by aprotinin. J Thorac Cardiovasc Surg 1990;100:520-527.

324. Bidstrup BP. Effect of aprotinin (trasylol) on aorto-coronary bypass graft patency. J Thorac Cardiovasc Surg 1993;105(1):147-153.

325. Allen S, Anastasiou N, Royston D, et al. Effect of aprotinin on vascular reactivity of coronary bypass grafts.. J Thorac Cardiovasc Surg 1997;113:319-326.

326. Sundt TM 3rd, Kouchoukas NT, Saffitz JE, et al. Renal dysfunction and intravascular coagulation with aprotinin and hypothermic circulatory arrest. Ann Thorac Surg 1993;55(6):1418-1424.

327. Dietrich W, Spath P, Ebell A, et al. Prevalence of anaphylactic reactions to aprotinin; analysis of two hundred forty-eight reexposures to aprotinin in heart operations. J Thorac Cardiovasc Surg 1997;113:194-201.

328. Blauhut B, Harringer W, Bettelheim P, et al. Comparison of the effects of aprotinin and tranexamic acid on blood loss and related variables after cardiopulmonary bypass. J Thorac Cardiovasc Surg 1994;108(6):1083-1091.

329. Salzman EW, Weinstein MJ, Weintraub RM, et al. Treatment with desmopressin acetate to reduce blood loss after cardiac surgery. A double-blind randomized study. N Engl J Med 1986;314:1402-1406.

330. Czer LSC, Bateman TM, Gray RJ, et al. Treatment of severe platelet dysfunction and hemorrhage after cardiopulmonary bypass: reduction in blood product usage with desmopressin. J Am Coll Cardiol 1987;9:1139-1147.

331. Tyers GF. Desmopressin: do we now know its role? Can J Surg 1990;33(1):5-6.

332. Seear MD, Wadsworth LD, Rogers PC, et al. The effect of desmopressin acetate (DDAVP) on postoperative blood loss after cardiac operations in children. J Thorac Cardiovasc Surg 1989;98(2):217-219.

333. Salzman EW, Weinstein MJ, Reilly D, et al. Adventures in hemostasis. Desmopressin in cardiac surgery. Arch Surg 1993;128(2):212-217.

334. Sloand EM, Alyono D, Klein HG, et al. 1-Deamino-8-D=arginine vasopressin (DDAVP)

increases platelet membrane expression of glycoprotein Ib in patients with disorders of platelet function and after cardiopulmonary bypass. Am J Hematol 1994;46(3):199-207.

335. Velders AJ, Wildevuur CRH. Platelet damage by protamine and the protective effects of prostacyclin: an experimental study in dogs. Ann Thorac Surg 1986;42:168-171.

336. Salzman EW. Hemostatic problems in surgical patients. In: Colman RW, Hirsh J, Marder VJ, Salzman EW, eds. Hemostasis and Thrombosis. Basic Principles and Clinical Practice. Philadelphia: JB Lippincott, 1992..

LEGENDS TO FIGURES

Figure 1. Normal platelet physiology. See text for explanation. β-TG, β- thromboglobulin; GP. glycoprotein; PF4, platelet factor 4; P-selectin, granule membrane protein 140; TSP, thrombospondin; vWF, von Willebrand factor.

Figure 2. Means ± SEM of the bleeding hme (corrected for skin temperature) in 87 patients undergoing isolated coronary artery revascularization at the Brockton/West Roxbury VA Medical Center. Blood samples were oblained in a subgroup of 24 patients. CBT,corrected bleeding time; CPB, cardiopulmonary bypass; Pre-CPB, before cardiopulmonary bypass; Pre-Hep, before the administration of heparin; Post-Hep, 5 minutes after the administration of heparin and before institution of CPB; min, minutes after the institution of CPB; Comp, at completion of CPB; Post-CPB, hours after completion of CPB.

Figure 3. The effect of CPB on activation-induced up-regulation of the platelet surface expression of P-selectin in whole blood *in vivo*. A standardized bleeding time was performed and, without touching the wound, the shed blood was collected with a micropipet directly from the wound site at 2 minute intervals (as shown on the horizontal axis) until cessation of bleeding. The sample was immediately anticoagulated and fixed, then analyzed by whole blood flow cytometry with the P-selectin-specific monoclonal antibody S12. This experiment is representative of 14 separate experiments. CPB 45: 45 minutes on CPB; post 2: 2 hours post CPB; post 24: 24 hours post CPB. From *Kestin, et al.* (19)

Figure 4. Effect of CPB on platelet reactivity to phorbol myristate acetate (PMA), as determined by whole blood flow cytometry. Peripheral blood samples were incubated with PMA 0 (circles), 0.25 (triangles), or 10 μM (squares). Panel A: platelet surface expression of P-selectin, as determined by monoclonal antibody S12. Panel B: platelet surface GPIb, as determined by monoclonal antibody 6D1. Data are mean ±SEM, n=16. From *Kestin, et al.* (19)

Figure 5. Effect of CPB on the platelet surface expression of the GPIb-IX and GPIIb-IIIa complexes. Panels A through D: the platelet binding of a panel of GBIb-IX-specific monoclonal antibodies, as determined by whole blood flow cytometry. The antibodies are directed against the von Willebrand factor binding site on GPIb (6D1), the thrombin binding site on GPIb (TM60), GPIX (FMC25), and the GPIb-IX complex (AK1). Panel E: ristocetin-induced binding of exogenous von Willebrand factor (vWf) to washed platelets, as determined by flow cytometry with a polyclonal anti-vWf antibody. Panels F through I: the platelet binding of a panel of GPIIb-IIIa-specific monoclonal antibodies, as determined by whole blood flow cytometry. Antibodies 7E3, 10E5, and M148 are directed against different epitopes near the fibrinogen site on the GPIIb-IIIa complex. Y2/51 is directed against the GPIIIa subunit. Panel J: binding site of exogenous radioiodinated fibrinogen to ADP-stimulated washed platelets. Fibrinogen binding is expressed as molecules x 10^3 per platelet. Data are mean ± SEM, n=4. From Kestin, et al. (19).

Figure 6. Shed blood thromboxane B_2 (TXB_2) level, skin temperature, and bleeding time measured simultaneously from the warm and the cold arm during and following hypothermic cardiopulmonary bypass (CPB) in 37 patients undergoing CPB surgery. One arm (Warm) was kept warm with a water-filled blanket set at 40°c while the other arm (Cold) was allowed to follow the systemic temperature. Hypothermia results in a significant prolongation of the bleeding time and a significant reduction in the TXB_2 level in the blood shed from the bleeding time site. ONBP, 20 minutes after institution of CPB; COMPBP, at completion of CPB. From *Valeri et al.*[20]

Figure 7. Perioperative plasma concentration of prothrombin fragment 1.2 (panel A), thrombin-antithrombin III complex (panel B), and fibrinopeptide A (panel C). P, post. CPB, cardiopulmonary bypass. Statistically significant changes from the preoperative value: *P<0.05; **P,0.01; ***P,0.001. (*Reproduced with modifications from Slaugheter et al Anesthesiology 1994;80:520-26*)

Figure 8. The median blood loss following the discontinuation of CPB and heparin reversal in 170 patients undergoing coronary artery bypass grafting (CABG, n= 144) and valve

replacement ± CABG (n=26). The average (±SD) age of the patients was 64.2 ± 9.5 yrs and the duration of CPB was 124.7 ± 46.5 minutes. The two bargraphs represent the two main components of post-CPB blood loss: OR, the component incurred intraoperatively after the normalization of the ACT, and SICU, the component incurred through chest drainage in the surgical intensive care unit.

Figure 9. Preoperative, intraoperative, and postoperative variables in terciles of the blood loss during the initial 4 hours post-cardiopulmonary bypass (post-CPB) in 76 patients undergoing valvular and coronary artery surgery. The tercile levels designated were low: 215-790 ml, n=26; medium: 805-1140 ml, n=26; and high: 1235-2515 ml, n=26. From *Khuri et al.*[18]

TABLE 1 Possible Causes of the Platelet Function Defect during Cardiopulmonary Bypass

Cause	Reference
Contact with synthetic surfaces and shear force	21, 26
Hypothermia	20
Lack of availability of platelet agonists (e.g. thrombin and ADP)	16, 34, 92
Heparin	177
Hyperfibrinolysis (plasmin and fibrinogen degradation products)	51
Denatured plasma proteins	21
Protamine	335
Aspirin	322
Nitrovasodilators	336
Cyanotic congenital heart disease and the state of oxygenation	51, 82

TABLE 2 Hematologic Changes Before, During, and After Cardiopulmonary Bypass (CPB) in Patients Undergoing Cardiac Surgery*

				After CPB		
Variable	n	Before CPB	Durimg CPB	2 h	24 h	48 h
Platelet Count (x 10^3/mm^3)	54	206 ± 7	104 ± 5	128 ± 6	123 ± 6	109 ± 6
Mean Platelet Volume (μm^3)	54	8.3 ± 0.2	7.7 ± 0.2	7.2 ± 0.3	8.0 ± 0.3	8.5 ± 0.3
Plasma β-TG (ng/ml)	54	59 ± 5	285 ± 29	291 ± 39	58 ± 5	59 ± 18
Plasma TxB_2 (pg/0.1 ml)	54	88 ± 9	91 ± 5	75 ± 7	59 ± 4	59 ± 4
Shed Blood TxB_2 (pg/0.1 ml)	54	571 ± 43	-	245 ± 30	405 ± 46	457 ± 37
Shed Blood 6-keto (pg/0.1 ml)	54	15.6 ± 2.0	-	30.7 ± 3.2	17.9 ± 3.6	13.4 ± 1.9
Hematocrit (vol %)	40	37.7 ± 0.5	21 ± 0.5	33.9 ± 0.8	30.8 ± 0.7	29.8 ± 0.5
Albumin (g/dl)	40	3.5 ± 0.1	1.8 ± 0.9	2.8 ± 0.9	3.0 ± 0.1	2.9 ± 0.1
Complement (mg/dl)	40	148 ± 6	61 ± 2	90 ± 4	80 ± 4	94 ± 4
Fibronectin (µg/ml)	40	450 ± 41	219 ± 11	318 ± 17	258± 12	273 ± 17
Immunoglobulin M (mg/ml)	40	136 ± 13	50 ± 5	81 ± 9	67 ± 7	66 ± 7
Immunoglobulin G (mg/ml)	40	977 ± 41	437 ± 21	681 ± 37	605 ± 32	579 ± 34
Fibrinogen (mg/dl)	40	357 ± 15	208 ± 10.6	266 ± 18	356 ± 17	511 ± 24
FVIII:C (%N)	40	109 ± 11	-	102 ± 9	121 ± 10	158 ± 12
FVIII-vWF (µ/ml)	40	1.62 ± 0.1	0.77 ± 0.1	1.77 ± 0.2	1.94 ± 0.2	2.65 ± 0.2
Plasminogen (%)	40	105 ± 3	56 ± 2	78 ± 2	67 ± 2	68 ± 2
Antithrombin III (%)	40	105 ± 2	60 ± 3	78 ± 2	74 ± 3	76 ± 2
D dimer (µg/ml)	22	0.6 ± 0.1	0.9± 0.17	3.0 ± 0.37	1.6 ± 0.18	1.1 ± 0.16

*Adapted from Khuri, et al. JTCvS 1992;104(1):94-107 (18). Changes within each row were significant by repeated measures analysis of variance (MANOVA).

β-TG, β-thromboglobulin; TxB_2, thromboxane B_2; FVIII:C, factor VIII clotting protein; FVIII-vWF, factor VIII-related antigen/von Willebrand factor. ** Denotes a significant difference from preoperative value by paired t test ($p<0.05$)

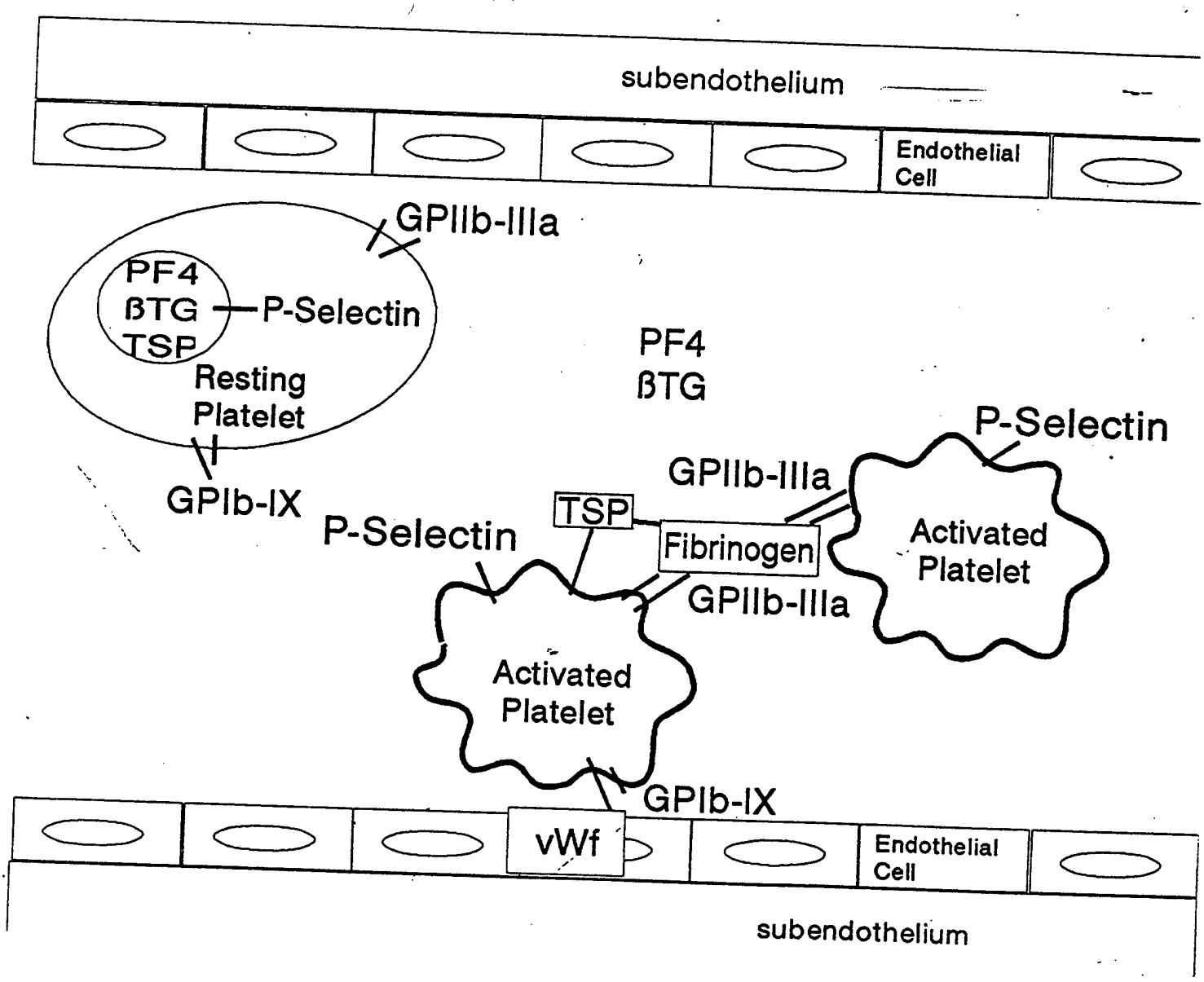
subendothelium
Endothelial Cell
GPIIb-IIIa
PF4
βTG
TSP
P-Selectin
Resting Platelet
GPIb-IX
PF4
βTG
P-Selectin
GPIIb-IIIa
TSP
Fibrinogen
GPIIb-IIIa
Activated Platelet
P-Selectin
Activated Platelet
GPIb-IX
vWf
Endothelial Cell
subendothelium

FIGURE 1

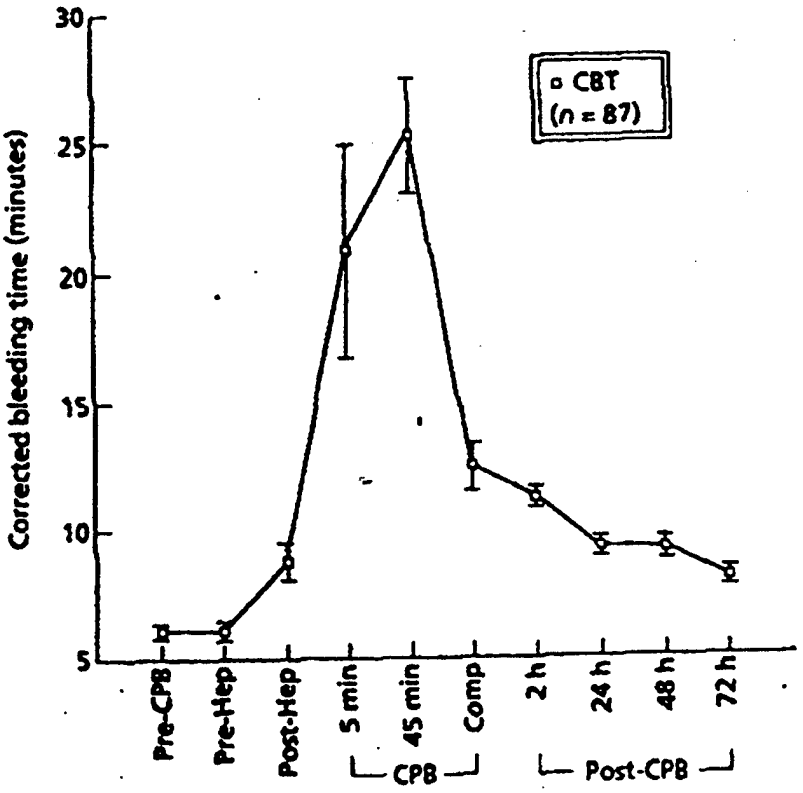
Corrected bleeding time (minutes)
30
25
20
15
10
5
CBT
(n = 87)
Pre-CPB
Pre-Hep
Post-Hep
5 min
45 min
Comp
2 h
24 h
48 h
72 h
CPB
Post-CPB

FIGURE 2

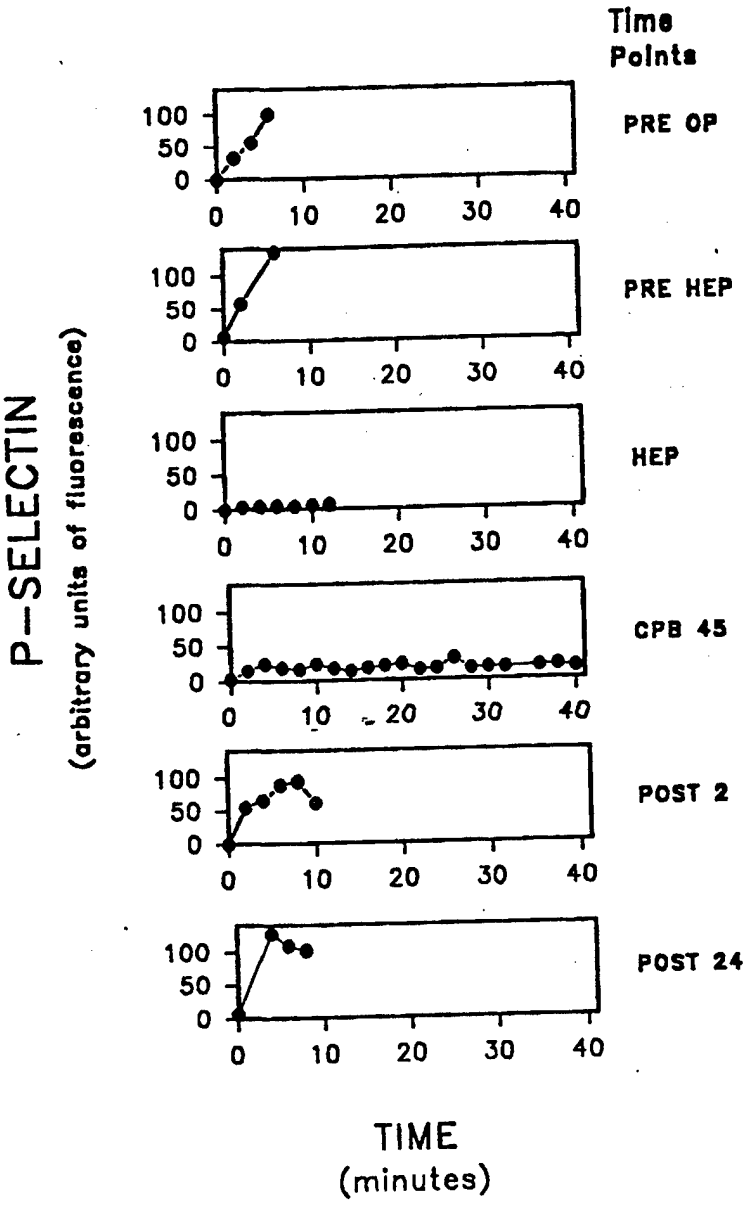
Time Points
PRE OP
PRE HEP
HEP
CPB 45
POST 2
POST 24
100
50
0
0
10
20
30
40
P-SELECTIN
(arbitrary units of fluorescence)
TIME
(minutes)

FIGURE 3

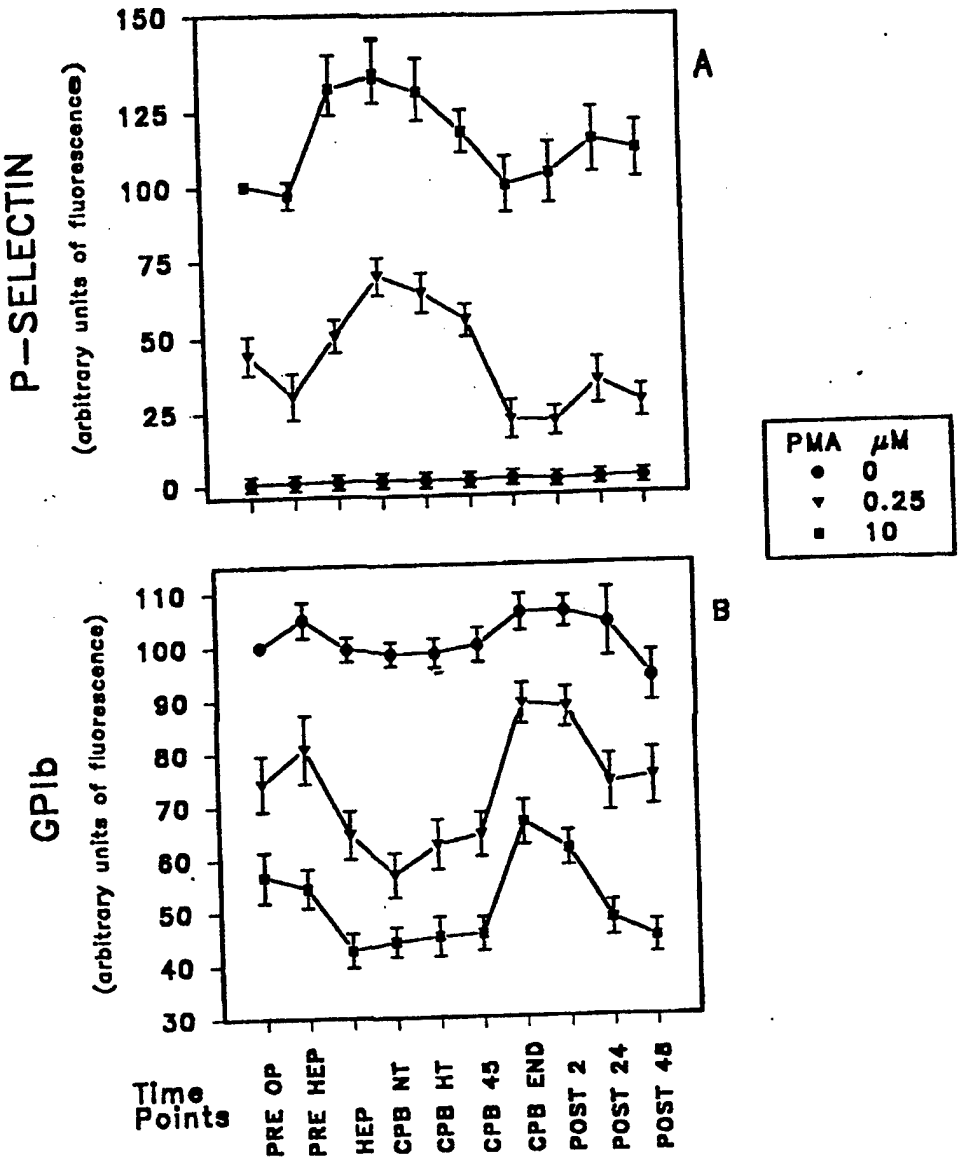
P-SELECTIN
(arbitrary units of fluorescence)
150
125
100
75
50
25
0
A
PMA μM
0
0.25
10
GPIb
(arbitrary units of fluorescence)
110
100
90
80
70
60
50
40
30
B
Time Points
PRE OP
PRE HEP
HEP
CPB NT
CPB HT
CPB 45
CPB END
POST 2
POST 24
POST 48

FIGURE 4

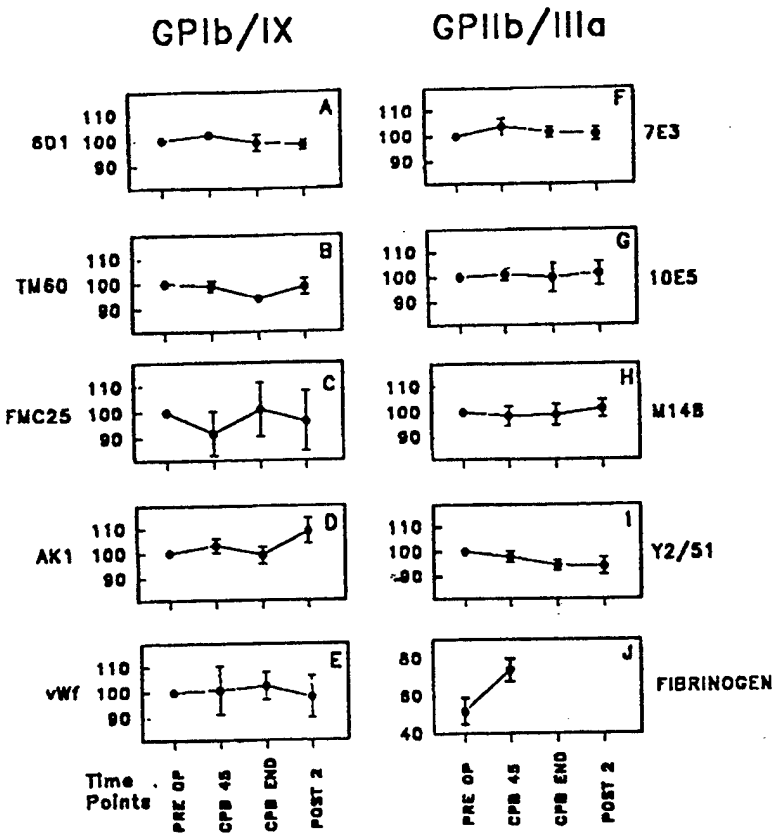
GPIb/IX
GPIIb/IIIa
A
6D1
110
100
90
F
7E3
B
TM60
G
10E5
C
FMC25
H
M148
D
AK1
I
Y2/51
E
vWf
J
FIBRINOGEN
80
60
40
Time Points
PRE OP
CPB 45
CPB END
POST 2

FIGURE 5

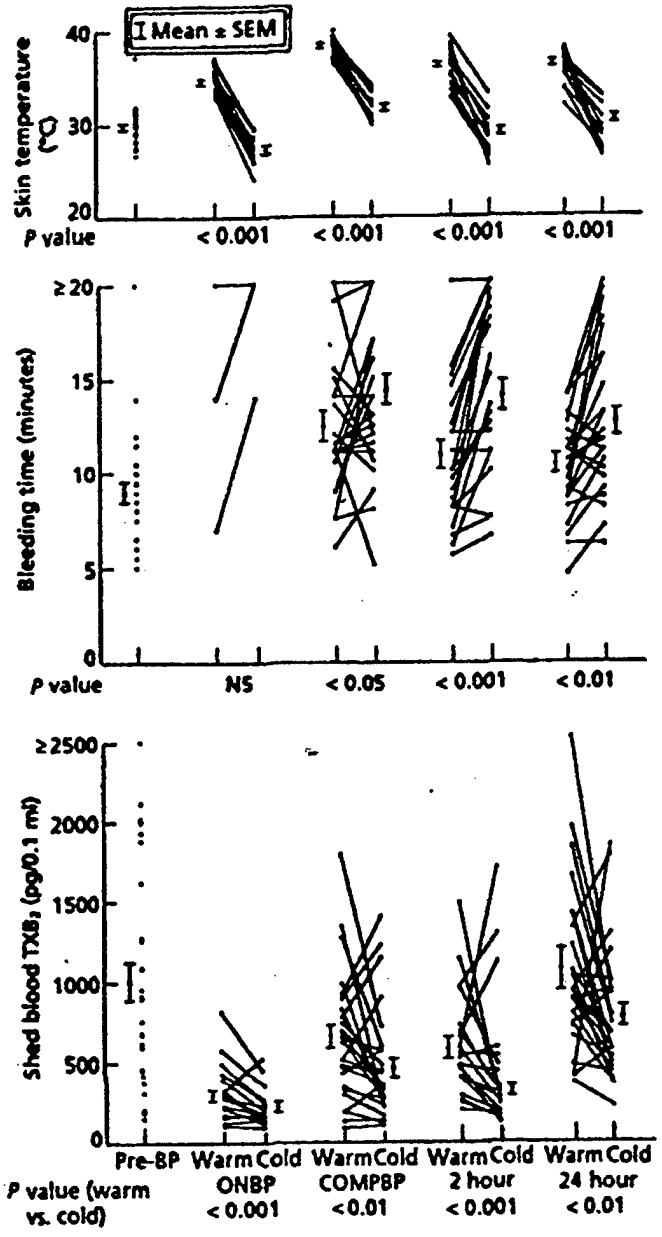
I Mean ± SEM
Skin temperature (°C)
40
30
20
P value
< 0.001
< 0.001
< 0.001
< 0.001
Bleeding time (minutes)
≥ 20
15
10
5
0
P value
NS
< 0.05
< 0.001
< 0.01
Shed blood TXB2 (pg/0.1 ml)
≥ 2500
2000
1500
1000
500
0
Pre-BP
Warm Cold
Warm Cold
Warm Cold
Warm Cold
ONBP
COMPBP
2 hour
24 hour
P value (warm vs. cold)
< 0.001
< 0.01
< 0.001
< 0.01

FIGURE 6

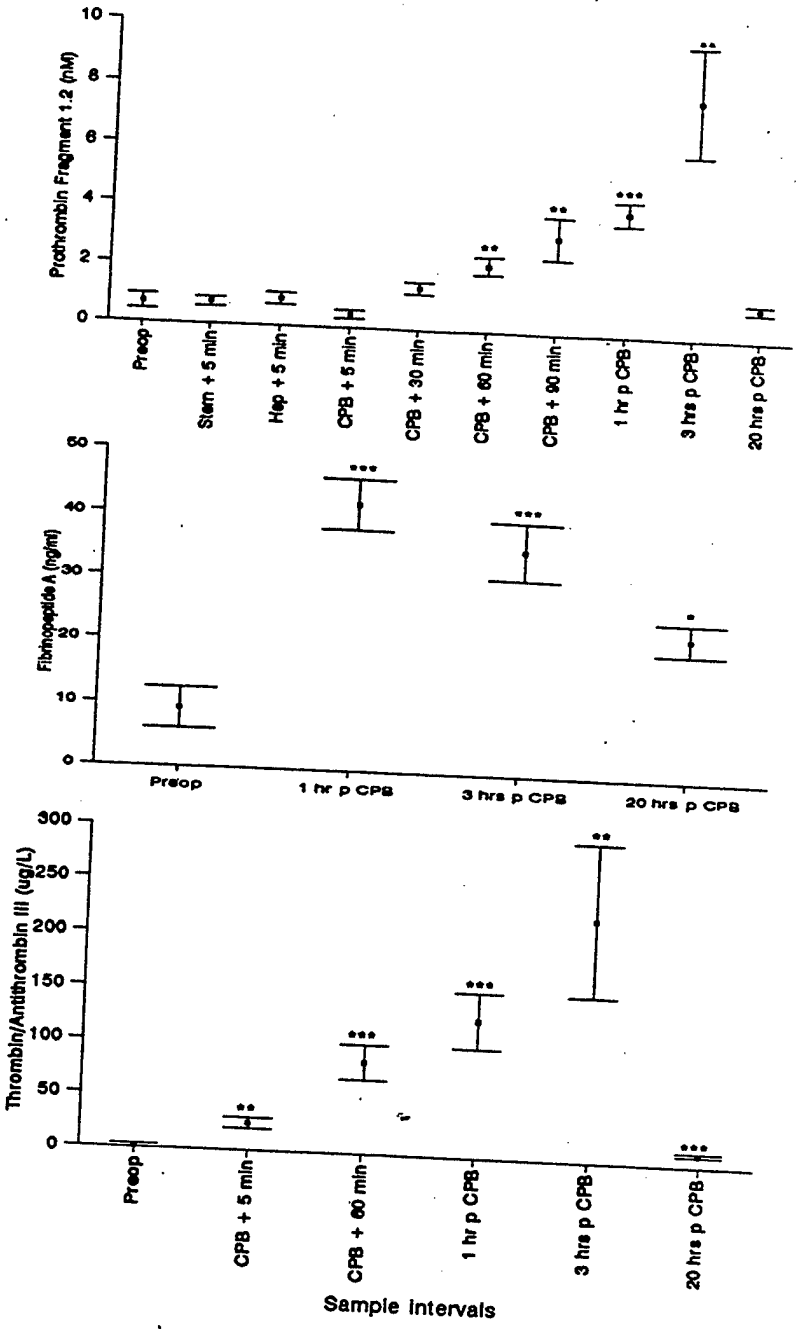
Prothrombin Fragment 1.2 (nM)
10
8
6
4
2
0
Preop
Stern + 5 min
Hep + 5 min
CPB + 5 min
CPB + 30 min
CPB + 60 min
CPB + 90 min
1 hr p CPB
3 hrs p CPB
20 hrs p CPB
Fibrinopeptide A (ng/ml)
50
40
30
20
10
0
Preop
1 hr p CPB
3 hrs p CPB
20 hrs p CPB
Thrombin/Antithrombin III (ug/L)
300
250
200
150
100
50
0
Preop
CPB + 5 min
CPB + 60 min
1 hr p CPB
3 hrs p CPB
20 hrs p CPB
Sample Intervals

FIGURE 7

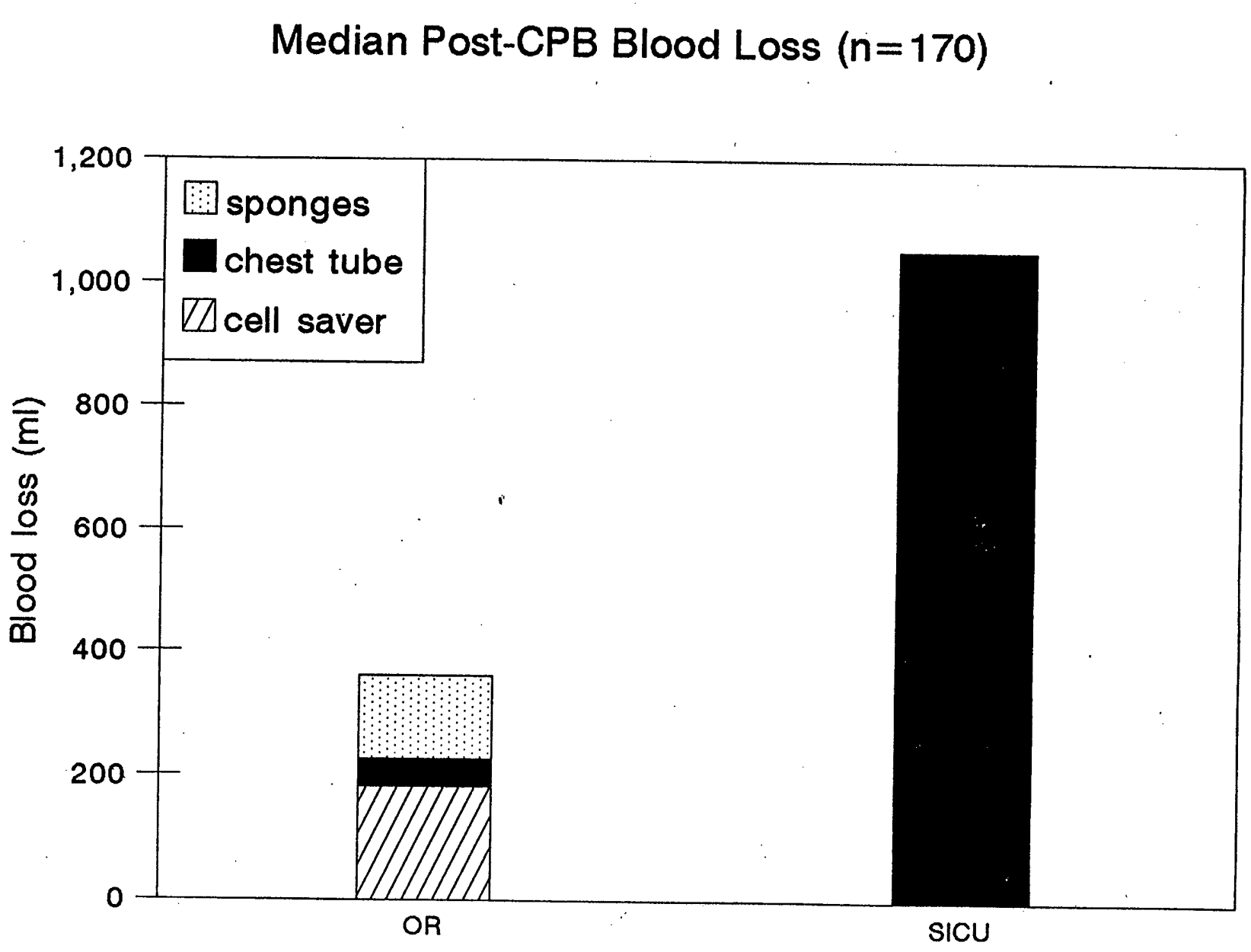
Median Post-CPB Blood Loss (n=170)
1,200
1,000
800
600
400
200
0
Blood loss (ml)
sponges
chest tube
cell saver
OR
SICU

FIGURE 8

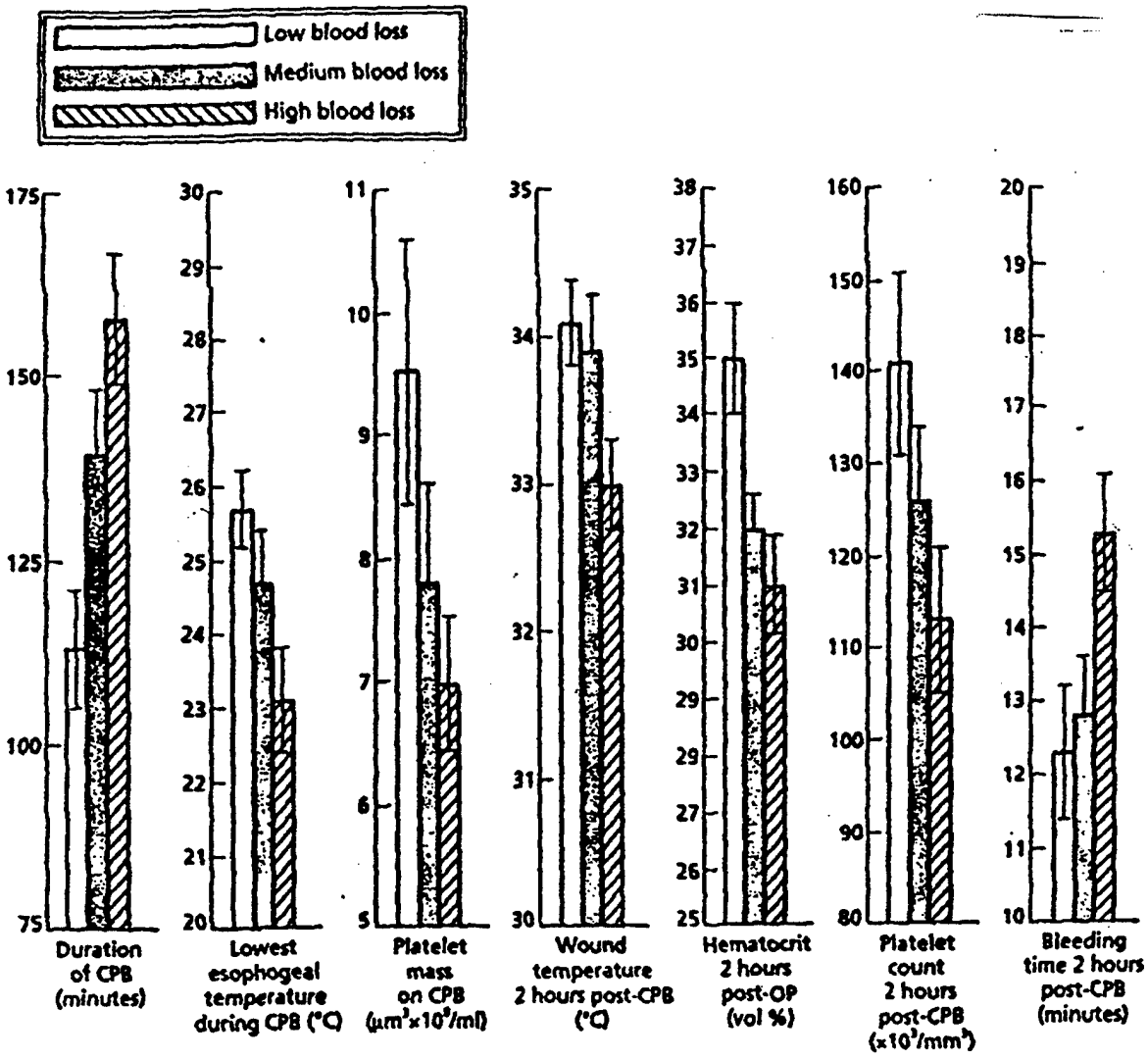
Low blood loss
Medium blood loss
High blood loss
175
150
125
100
75
Duration of CPB (minutes)
30
29
28
27
26
25
24
23
22
21
20
Lowest esophogeal temperature during CPB (°C)
11
10
9
8
7
6
5
Platelet mass on CPB (μm³×10⁹/ml)
35
34
33
32
31
30
Wound temperature 2 hours post-CPB (°C)
38
37
36
35
34
33
32
31
30
29
27
26
25
Hematocrit 2 hours post-OP (vol %)
160
150
140
130
120
110
100
90
80
Platelet count 2 hours post-CPB (×10³/mm³)
20
19
18
17
16
15
14
13
12
11
10
Bleeding time 2 hours post-CPB (minutes)

FIGURE 9